

Degree in Mathematics

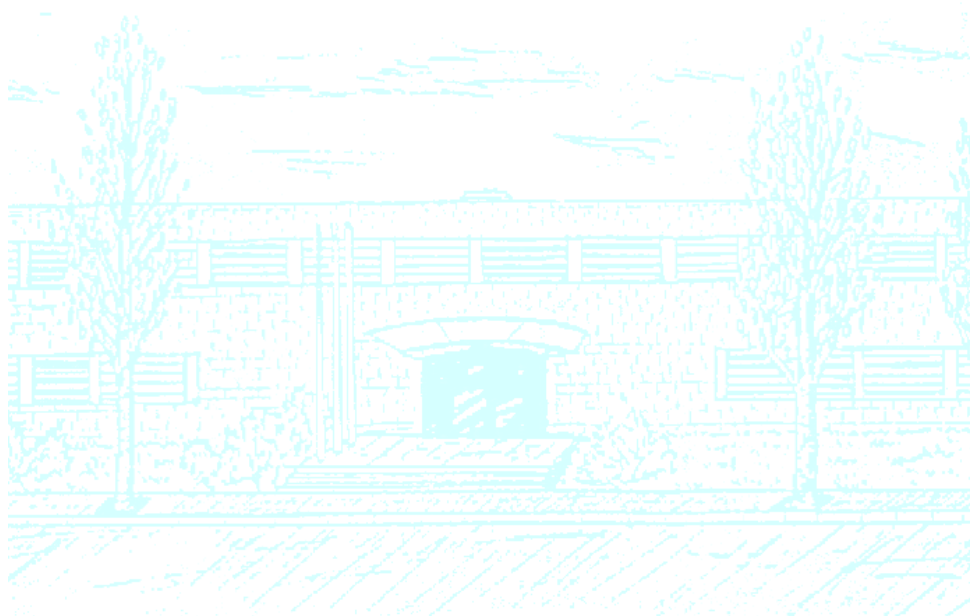
Title: Cardiopulmonary responses to exercise in altered gravity environments

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Academic year: 2017/18



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ACKNOWLEDGEMENTS

In the first place, I would like to offer my special thanks to my advisor Ana Diaz. You have given me this amazing opportunity and taught me everything I know about bioastronautics. Thank you for trusting me, for your unconditional support and for your contagious passion. I couldn't have imagined a better guide through this incredible journey.

I would also like to express my gratitude to my lab partner Francisca. Thank you for sharing the early wake-ups, the heavy platform lifting and the stress during the experiment failures. Even if my Spanish lessons haven't been really fruitful, you have been the best English teacher.

A sincere thank you to all the subjects who participated in the study, for their enthusiasm and selfless willingness to help (Especially to the ones who had to do extra sessions, sorry about that!). It has been really easy and fun to work with all of you.

And last, but not less important, to my family and friends. You have always believed in me, encouraged me and made me who I am today. I'm so grateful to have all of you by my side.

Finally, my research visit at EEUU has been possible thanks to the financial support of the TFG scholarship from CFIS and Fundació Cellex, the MOBINT scholarship from Gerneralitat de Catalunya and Cornell University. The project has also been partially funded by the NYSG faculty grant.

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To my mum

1. INTRODUCTION

As the limits of human exploration expand farther into space, there becomes an increasing need to condition and maintain the human body for life beyond our planet. Astronauts on space missions experience various detrimental physiological effects including (but not limited to) muscular atrophy, diminished cardiopulmonary function, and redistribution of internal fluids. These changes can lead to orthostatic intolerance and diminished exercise capacity while back on Earth [1]–[4]. Space medicine has been studying all these effects associated with space exploration since the very beginning of space missions, improving and developing new countermeasures to effectively reduce health risks. However, with new missions to Mars and the Moon on the horizon, greater knowledge and better countermeasures are crucial to guaranteeing the operational efficiency of the crew members and, consequently, the success of the missions.

Artificial gravity combined with exercise has been proposed as a multi-system countermeasure given that it presents the potential to counteract the detrimental effects of weightlessness in several of the body's systems at once [5]. Nevertheless, the ideal gravity conditions under which this exercise should be performed to be the most effective are still unknown. After the NASA workshop on “Research and Operational Considerations for Artificial Gravity Countermeasures” held in February 2014, the need of an international Ag roadmap was highlighted but, it wasn't until 2017, when it was finally established [6].

The Ag roadmap determines the current gaps in knowledge and creates a framework to translate abstract ideas into concrete research activities needed to fill these gaps. One of the main focuses is to obtain dose-response curves representing the relationship between gravitational dose and physiological response. These curves are key to determine the Ag range where the systems in the body operate closest to the ones in Earth. Another important task involves determining the physiological responses at Martian gravity levels. Different partial-gravity analogs have been outlined as important approaches to achieve both of these objectives including parabolic-flights, water immersion and head-up tilt [6].

This research effort tries to start bridging these gaps of knowledge and generate dose-response curves of musculoskeletal, cardiovascular and pulmonary systems between 0g

and 1g. To accomplish this, a human experiment was designed and executed to investigate acute physiological responses to partial G with and without exercise. A tilting platform was built and used to simulate several gravitational environments in the head-to- toe direction (Gz) by tilting the bed to the appropriate tilt angle. Additionally, Mars gravity (0.38 g) was chosen as one of the tested Ag conditions since it is highly pertinent to current missions and future planetary surface exploration.

2. BACKGROUND

2.1. PHYSIOLOGICAL DETRIMENTAL EFFECTS IN SPACE

Human spaceflight tests astronauts' physiology with a completely new and hazardous environment. Over time, the human body has adapted to Earth's gravitational conditions. When these conditions change, as occurs in microgravity, all our systems try to adapt to ensure their correct functioning in the new weightless environment. Thus, several physiological changes take place, including bone loss, muscle atrophy, cardiovascular deconditioning, and neurovestibular adaptation [7][8].

Skeletal remodeling depends on the stress being applied to the bones. In microgravity conditions this stress, usually caused by the skeletal loading when walking or running, is reduced. Consequently, the skeletal remodeling process is much slower, and astronauts might lose around 1-2% of bone density per month [8][1][9]. Due to the bone demineralization, a 60-70% increase in urinary and fecal calcium can be detected during the first days of the space mission [7][1]. These physiological changes could negatively affect the crew members' health increasing the risk of bone fracture and kidney stone formation. Low light levels and high carbon dioxide concentrations, factors present in the ISS, could also increase bone loss[1].

In weightless conditions our muscles do not need to work as hard as on Earth to maintain an upright posture. This decrease in the activity of postural muscles leads to a decrease in their strength and mass. Studies show an up to a 20% muscle mass loss after a 2-weeks space flight [8]. These adaptations cause muscle soreness when the crew return back to Earth when their muscles are again loaded with gravitational forces. Other factors that can diminish muscle mass are suboptimal nutrition and stress [2][10].

Cardiovascular changes in microgravity are mainly caused by the redistribution of body fluids. Right after entering into a microgravity environment, the fluid shift phenomenon takes place, reducing about 10% the fluid volume in the lower body, anecdotally known as 'puffy face–bird leg' syndrome [11]. The increase in the arterial blood pressure that comes with the upper body blood volume increase, triggers the cardiopulmonary system leading to a reduction of the total blood volume up to a 11% during the first 24 hours of

the space flight [8]. Additionally, due to the lack of gravity the heart doesn't have to work as hard to pull the blood into the head and consequently heart atrophy occurs. An 8-10% cardiac muscle decrease has been observed in post-flight studies [3]. The main issue with these adaptations comes when astronauts come back to Earth and all their blood is pulled down by the gravity forces. Due to the diminished cardiac function and the reduced blood volume they could experience orthostatic intolerance, being unable to stand for 10 consecutive minutes [12].

Finally, the neurovestibular system also adapts to weightless conditions normally during the first two days of space flight causing several symptoms such as pallor, cold sweating, nausea and, in some cases, vomiting. The term space motion sickness has been used to describe the symptoms related with the neurovestibular acclimation [4]. This phenomenon also occurs in the inverse situation when returning to Earth, being more noticeable after long-term missions [13].

Apart from these changes associated with the lack of gravity, there exist other hazards that can put crew members' health at risk. Radiation exposure is one of the most important ones. Outside the Earth's atmosphere we no longer have protection against space radiation and astronauts are exposed to much higher levels of ionizing radiation. High-dose exposure can also happen when performing extravehicular activity. Immediate deterministic effects include nausea, vomiting and even organ failure due to the inability of the cells to reproduce. Low-dose exposure produces long term effects including central nervous system damage, cataracts, reduced fertility and cancer risk.[14] However, the cancer risk associated with this kind of exposure is really difficult to predict due to the large uncertainties associated with the projected cancer risk estimates [15].

Another major hazard is the isolated confinement crew members have to live in and all the psychological effects this can cause, such as fatigue, sleep debt, emotional effects, or stress. All these factors can easily lead to disputes that could potentially have a major impact on the success of the mission.[16]

Since the Apollo era, scientists and engineers have studied these physiological changes associated with human spaceflight and exploration. Bioastronautics has lead to several key medical discoveries and to the development of countermeasures against the

detrimental effects of weightlessness. However, the further and longer we want to explore, the more challenges space medicine will have to overcome.

2.1.1. Insight on the cardiovascular system in microgravity

The main objective of the cardiovascular system is to transport nutrients to body tissues. Consequently, blood flow is regulated by nutrient demand of our body. Several control mechanisms act within the heart and blood vessels to achieve the required cardiac output and arterial pressure and ensure the properly functioning of the system. Figure 1 shows the different mechanisms as well as their reaction times and feedback gains.

Short-term mechanisms act within seconds or minutes and these include the baroreceptor and chemoreceptors mechanisms and the central nervous system ischemic response. They rapidly react to a drop in arterial pressure by increasing heart rate and contractility of the heart and contracting the veins and the peripheral arterioles. These changes will lead into an increased Heart Rate, Stroke Volume and Total Peripheral Resistance, which will increase mean arterial pressure ($MAP = HR \cdot SV \cdot TPR$). An increase in blood pressure would cause the opposite reaction, therefore maintaining normal blood pressure levels.

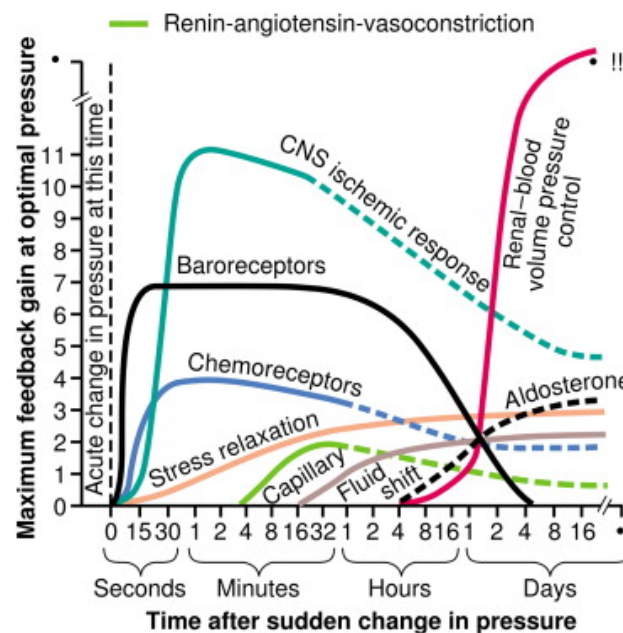


Figure 1. Gain and time of intervention of different arterial pressure control systems [17].

Medium-term mechanisms are necessary after a few minutes when the short-term ones adapt to the new conditions and become less sensitive. These include stress relaxation, renin-angiotensin-vasoconstriction and capillary fluid shift mechanism.

If the blood pressure continues to be out of their normal range after 24 hours, long-term mechanisms respond by increasing the urine output and consequently decreasing blood volume and thus blood pressure. This mechanism is called Renal-blood Volume Pressure control and plays an important role in microgravity cardiovascular regulation [18].

The effect of these mechanisms in controlling blood pressure can help to explain cardiovascular responses in microgravity. As it was explained before, right after leaving Earth's gravity the body fluids shift into the upper body (See figure 2). Blood volume experiences a redistribution in the body increasing the arterial pressure at chest and head levels. In early-flight stages heart size and stroke volume increases while heart rate decreases due to the short-term mechanisms [19].

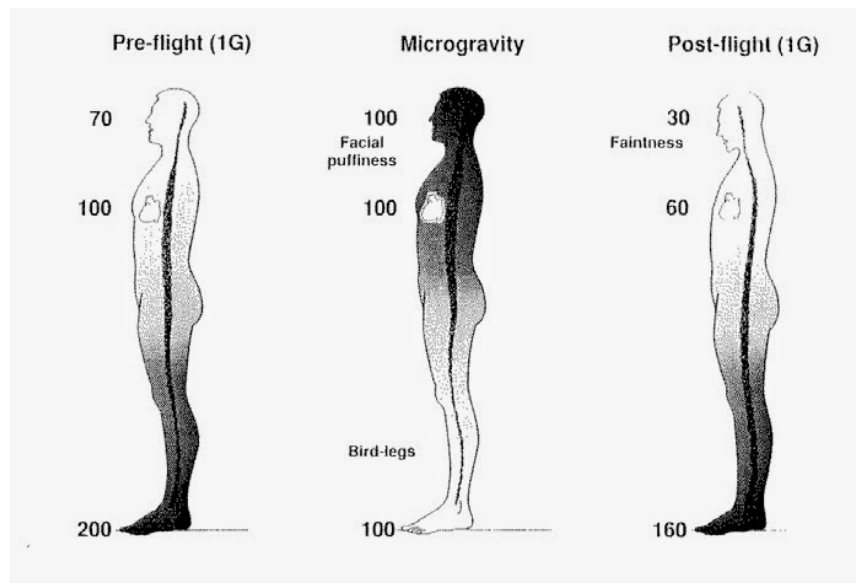


Figure 2. Blood distribution and pressures measured pre-flight, in microgravity and post-flight [20].

After the first day in orbit, plasma volume drops up to 17% of its pre-flight value. However, urine output doesn't seem to increase [3]. This contradictory result can be attributed to the movement of the albumin-containing fluids from the intravascular to the extravascular space [21]. Plasma volume reduction induces a reduction in red blood cell mass, producing a net effect of a 11% of total blood volume loss in space.

Blood volume reduction together with a reduction of the heart rate stimuli during space flight also reduces heart activity and its size. Cardiac rhythm disturbances have also been observed in astronauts with the cause still being unknown [3].

Although cardiovascular adaptation does not lead to major problems while in space, several drawbacks of this adaptation are noticeable when astronauts return to the Earth's environment. The reduced blood volume is pulled down again by gravity forces, as can be seen in Figure 2, drastically reducing blood pressure at the head level. The diminished cardiac function in combination with the vasoconstriction limitations does not contribute to restore normal pressure levels and orthostatic intolerance occurs [3].

Some of the aspects of the cardiovascular system in space are still unknown, especially regarding how they continuously develop under reduced gravity conditions. More research is needed in order to understand and manage these cardiovascular effects to ensure crew members' health and effective performance during and after flight.

2.1.2. Insight on the pulmonary system in microgravity

The respiratory system works closely together with the cardiovascular system to ensure enough oxygen is delivered to our tissues. Therefore, pulmonary activity is determined by the metabolic consumption of our body. Despite being one of the lower density organs, the highly vascularized structure of the lung, with 1500 miles of airways and 600 miles of capillaries, makes it a compliant structure that deforms under its own weight. The influence of gravity on the lung is therefore not trivial and new breathing patterns must be adopted in space to meet the same oxygen demand. Two models can be used to better understand pulmonary regulation: The zone model and the slinky analogy.

The zone model of pulmonary perfusion was historically discovered by J.B. West in 1964 [22]. It establishes that gas exchange between alveoli and capillaries is regulated by the interaction of three main pressures: Arterial pressure(P_a), venous pressure(P_v) and alveolar pressure(P_A). On Earth, the presence of gravity forces create a hydrostatic gradient on the arteries and veins increasing P_v and P_a when moving from the top to the bottom of the lung. However, this gradient in pressure cannot be seen in alveolar air leading to three possible situations (Figure 3A):

- $P_A > P_a > P_v$: *Zone 1*. Alveolar pressure is higher than the arterial and venous pressures and consequently, the capillaries collapse, occluding the blood flow.
- $P_a > P_A > P_v$: *Zone 2*. Only arterial pressure is higher than the alveolar pressure creating a Starling resistor. The blood flow is then determined by the difference between arterial and alveolar pressures.
- $P_a > P_v > P_A$: *Zone 3*. Vascular pressures are higher than the alveolar pressure and blood flow is regulated by the difference between arterial and venous pressure.

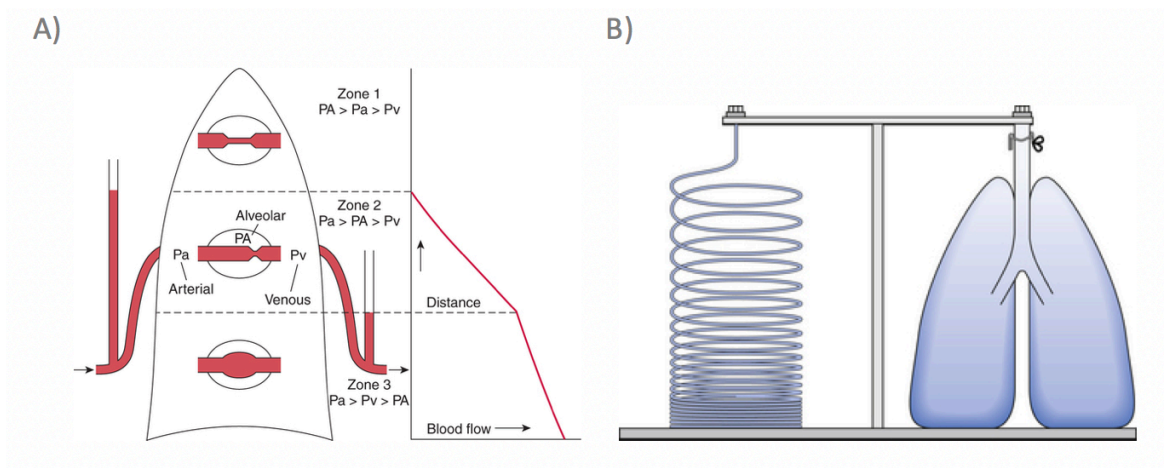


Figure 3. A) Representation of the three lung zones according to the zone model of pulmonary perfusion. B) Slinky analog to explain alveolar size distribution in the lungs [23][24].

In order to model the effect of the lungs' own weight gradient on the alveolar size we can think about the lung as a slinky (Figure 3B)[25]. Gravity causes the slinky to slump under their own weight, distributing the upper coils wide apart and the ones in the bottom close together. The same distribution can be seen in the alveoli, therefore being the alveolar size greater in the upper part of the lung than in the lower part. This model also enables us to predict blood flow distribution imagining it as flowing through the material of the slinky itself. Under microgravity conditions, this model would predict a more uniform alveolar size as well as blood flow distribution along the lung.

Once these two concepts are clear, we can think about how gravity would affect the pulmonary system. Lung volumes and capacities described in Figure 4 are normally used to characterize breathing mechanics.

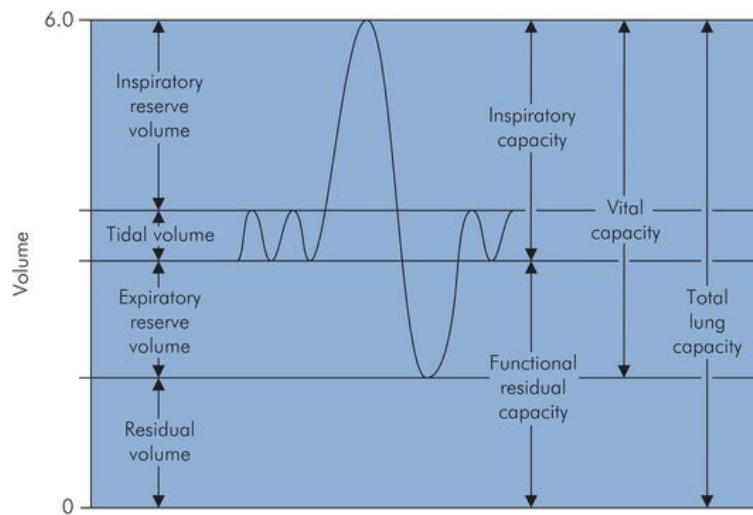


Figure 4. Respiratory excursions during normal breathing and during maximal inspiration and minimum expiration [26].

Studies in Skylab have shown a decrease in Vital Capacity (VC) during the firsts days of flight, returning to pre-flight levels after the fourth day. This temporary change is presumably due to the shift of the blood to the upper body in early flight, which causes a greater chest pressure. Both Expiratory Reserve Volume (ERV) and Residual Volume (RV) decreased in microgravity and consequently, so did the Functional Residual Volume (FRV). ERV reduction can be explained by the decrease in inspiratory forces due to the lower contribution of the abdominal weight in microgravity. For its part, RV decrease is more related to the more uniform distribution of the alveolar size (Slinky model), allowing a better overall distribution of the alveolar air [27].

When studying the breathing patterns, Tidal Volume (TV) also decreased about 15% in microgravity with a consequent increase in the Respiratory Frequency of 9% (Rf). This increase in the respiratory frequency seems to be a compensatory response to maintain minute ventilation at the needed levels ($VE = TV \cdot Rf$). However, the causes of the TV decrease are still unclear [27]. Overall, VE decreased 7%, with no changes in oxygen consumption or carbon dioxide production being detected (same metabolic needs). This reduction in the total ventilation was not accompanied by a decrease in alveolar ventilation suggesting a more efficient gas exchange due to the reduction of the dead space. If we think about the slinky model, the more uniform distribution of the blood flow presumably decreases dead space increasing alveolar exchange [28]. Figure 5 illustrates the comparison between Earth and microgravity breathing mechanics.

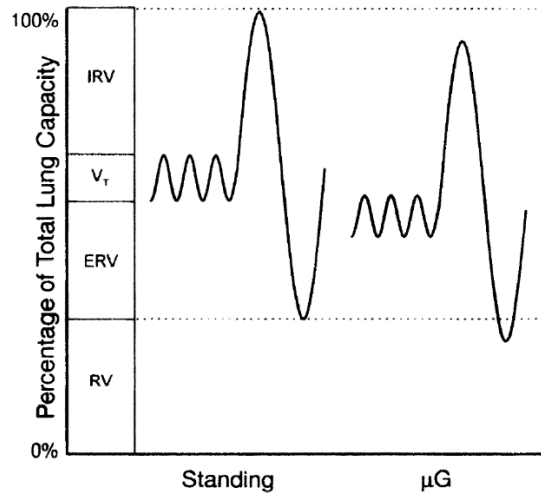


Figure 5. Lung volumes and capacities under Earth and microgravity conditions.[27]

Although the adaptation of the pulmonary system to different gravity environments does not lead to major health concerns, it provides a window to the cardiovascular system changes. Moreover, its interaction with the cardiovascular system give us important information about physiological fitness indicators such as VO_2 max, which is crucial when performing tasks in space or another planetary surface.

2.2. CURRENT COUNTERMEASURES

Over more than 50 years of space flight several countermeasures have been developed to mitigate the detrimental physiological effects of being in space. Since the adaptation of the body leads into many health problems when returning to Earth, the main objective of these countermeasures should be to maintain the body as close to normal gravity conditions as possible. Countermeasures are usually focused on mitigating only one of the effects of space flight (local countermeasures). Others intend to mitigate several issues at once (multisystem countermeasures), with the latter being the most interesting to study.

Some local countermeasures include: Negative pressure suits and isotonic fluid taken orally for fluid shift effects, diet supplemented with calcium and vitamins D and K for bone demineralization, dietary supplementation with amino acids for muscle atrophy, antinauseant medications (promethazine, scopolamine) for motion sickness, etc.[7]

The most important multisystem countermeasures include aerobic and resistive exercise. Artificial gravity and the combination of exercise and Ag are potential countermeasures to be implemented in the future.

2.2.1. Aerobic and resistive exercise as a countermeasure

Aerobic exercise can currently be performed on the ISS using a cycle ergometer or a motorized treadmill, while ARED (Advanced Resistive Exercise Device) is used for resistive exercise [29]. Exercising helps to maintain muscle strength as well as to decrease calcium loss through the loading forces generated, however, despite major improvements in the exercise routines, exercise alone has not consistently maintained pre-flight bone density levels [30]. Exercising in microgravity also helps to maintain the cardiovascular system closer to pre-flight conditions by providing cardiovascular stimuli similar to the ones on Earth. Yet, some crew members still experience orthostatic intolerance. Exercise combined with artificial gravity is a potential solution for obtaining more efficient countermeasures. Apart from the physical benefits, in a confinement environment exercising diary helps to maintain mental health and to decrease negative psychosocial effects [31].



Figure 6. Astronaut exercising with the ARED device onboard the ISS [NASA]

2.2.2. Artificial gravity as a countermeasure

Artificial gravity consists of exposing a person to a simulated gravity environment. To do so, it is necessary to create forces equivalent to the gravitational ones. Both linear and centrifugal accelerations are valid techniques, however, steady rotation is a more realistic option in terms of the space needed to create Ag for an extended period of time. The idea of generating a gravitational environment in space has been explored since the beginning of space exploration. In 1973 Korolev proposed a rotating space station composed of two modules that provide a continuous Ag of 0.16 G [32]. Even though this model is desirable for maintaining crew member's overall health, it has been discarded due to its large mass, complex design, and expensive cost. Alternatively, intermittent exposure to Ag has been proposed as a more feasible option. A short-radius centrifuge can be used on the ISS or future space stations to provide short periods of gravity loading to mitigate the detrimental effects of microgravity.

Some studies have shown the efficiency of intermittent Ag in mitigating orthostatic intolerance [5]. Furthermore, other studies have also shown that it could be useful to maintain normal cardiovascular control, however it would be insufficient at preventing a decrease in exercise capacity or cardiac remodeling [33][34]. Even if theoretically it could be useful to mitigate skeletal and muscular effects, some of its effects still remain unknown. Some limitations associated with Ag are the motion sickness side effects and the fact that if the Ag level needed is too high it could lead to orthostatic intolerance in passive subjects. This is why Ag combined with exercise has been proposed as a better approach than artificial gravity on its own. [31]

2.2.3. Artificial gravity combined with exercise as a countermeasure

According to recent ground-based studies, exercise performance under an artificial gravity environment is currently the most promising multisystem countermeasure [35][36]. It would theoretically solve the problems of Ag and exercise individually, restoring normal bone density mechanisms as well as increasing muscle sympathetic activity, normal cardiovascular activity, and preventing orthostatic intolerance.

However, the optimal parameters needed to maximize the beneficial effects of this countermeasure are still unclear. What is the minimum Ag level needed to protect from physiological deconditioning? What is the optimal exposure time? What kind of exercise protocol would give better results and at what workload levels? Further investigation is required to find answers to all these questions.

2.3. SPACEFLIGHT ANALOGS

Spaceflight analogs offer a valuable insight into the physiological responses over different gravitational conditions without the need of a real microgravity exposure. They provide us with an option to test the different countermeasures with a greater number of subjects and in a less expensive way than actual spaceflights. Although analogs might not replicate all aspects of microgravity, they can simulate just one or several characteristics of spaceflight exposure. In this section we are going to focus on the analogs capable of simulating fluid shift, which are more useful for determining the cardiovascular and pulmonary responses.

2.3.1. The importance of partial-gravity analogs

As future space missions include exploring new planetary surfaces like Mars, a better understanding of how the astronauts' physiological outcomes develop in partial-gravity is needed. Partial-gravity analogs would enable us to create dose-response curves of the relationship between physiological responses and G level (Figure 7).

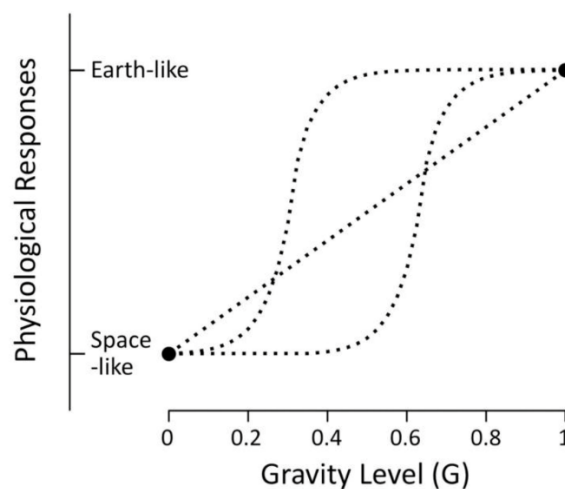


Figure 7. Hypothetical dose-curve responses between 0 and 1 gravity levels [37].

These dose-response curves are essential for determining if Moon or Mars gravity levels are enough to protect the crew’s health from physiological deconditioning and for establishing the artificial gravity level needed to mitigate the detrimental effects of weightlessness. They would also be used to determine the optimal parameters for Ag protocols and prescriptions during space missions such as the artificial gravity level needed and the duration of exposure.

2.3.2. *Partial-gravity analogs to simulate fluid shift*

Some space analogs that enable us to simulate fluid shift under different partial-gravity conditions are parabolic flight, head down-tilt (HDT), head up-tilt (HUT) and short-radius centrifugation.

Aircraft parabolic flights repetitively simulate short-periods of altered-gravity environments by changing altitude and speed while following a parabolic pattern (Figure 8). By changing the pitch angle, different Ag levels can be achieved as for example microgravity (47°), Moon (42°) or Mars (38°) [38]. The main drawback of this analog is the short exposure time, typically around 20 seconds when simulating microgravity, which restricts the parabolic flight studies to short-term responses.

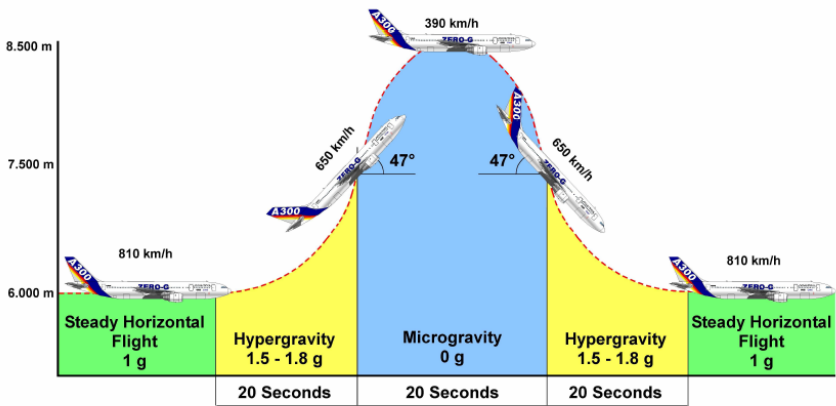


Figure 8. Parabolic flight trajectory and gravity level over time.

Bed rest has been the more used space analog, including both head down-tilt and head-up tilt. In this analog subjects lay on a bed or platform tilted at a specific angle with respect to the horizontal plane. The breakdown of the gravity force in the head-to-toe

direction and its perpendicular enables us to simulate different gravity levels such as the Martian (0.38 g) or the Lunar ones (0.16 g) using 22.4° HUT and 9.5° HUT respectively. Microgravity can also be simulated using -6° HDT [39]. However, the perpendicular component of the breakdown causes tissue compression due to its weight. This is not present in real microgravity and is a limitation of the simulation.

Short-radius centrifugation uses the inertial forces generated during rotation to simulate gravity forces. Centrifugal forces depend on the radius and the angular velocity (Equation 1). Changing these parameters allows for different gravity environments to be obtained.

$$F_{\text{centrifugal}} = m \cdot \omega^2 \cdot r \quad (1)$$

The radius refers to the distance from the object to the rotation center. Hence, since in a short-radius centrifuge paradigm the subject is positioned radially with his head close to the rotation center, the centrifugal force would be greater in his feet than in his head. It is important to quantify the effects caused by this gravity gradient since they would not happen in constant gravity environments and this difference must be taken into account.



Figure 9. NASA-provided Short Radius Centrifuge at UTMB in Galveston, Texas [NASA].

3. RESEARCH GAPS AND OBJECTIVES

During the “Research and Operational Considerations for Artificial Gravity Countermeasures” Workshop held at the NASA Ames Research Center in February 2014, the main gaps in knowledge on how to implement Ag in a space vehicle were discussed. Clément published in 2017 a paper identifying these gaps and established an international Ag roadmap [6]. The five main gaps are:

- *GAP 1*: The minimum AG level required to mitigate the effects of microgravity.
- *GAP 2*: The minimum AG duration required to mitigate the effects of microgravity.
- *GAP 3*: The potential effects of Mars gravity.
- *GAP 4*: The health consequences of Coriolis, cross-coupled acceleration, and gravity gradient.
- *GAP 5*: If the AG prescription determined during ground-based studies in humans will be effective, acceptable, and safe for the crew in space.

The present study focuses on two of the mentioned gaps: Gap 1 and Gap 3.

3.1. Minimum Ag level required to mitigate the effects of microgravity

After more than 60 years of spaceflight we still don't have the necessary knowledge of how our physiological systems react and adapt to different gravity levels. Dose-response curves will provide a useful insight into how our systems develop with different G doses. This information is key when trying to determine the Ag needed to prevent weightless deconditioning.

The effect of Ag levels between 0g and 1g in humans have been tested in ground-studies using several partial-gravity analogs. Parabolic-flight has recently been used to study acute responses of the cardiovascular, ocular and sensorimotor systems. Some experiments have already been carried out during the first joint European partial-g parabolic flight campaign using the Airbus A300 ZERO-G to simulate microgravity, Moon and Mars gravity levels [38]. These included, among others, some cardiovascular related studies such as the study of alterations in autonomic cardiovascular control [40]. Additionally, new parabolic-flight campaigns are scheduled for Spring 2018.

Bed rest simulations have been used for more than 30 years to study the physiological effects of spaceflight [39]. This analog has been commonly adopted to study long term responses with regard to orthostatic intolerance, blood volume, aerobic power, psychoneuroendocrine alterations or bone loss [41] [42] [43]. However, in contrast with head-down bed rest, which has been widely studied, head-up tilt has not been as popular for studying partial-gravity and, consequently, few studies simulating G levels between 0g and 1g are available [44].

There exist other partial-gravity analogs that allow for physiological responses to be studied while performing exercise such as the suspension techniques or lower body positive pressure techniques [45]. Some of these analogs have been used to conduct suit tests as the NASA Space Vehicle Mockup Facility's partial gravity simulator (POGO) which is equipped to measure kinematics and metabolic rates [46].

Finally, several computer models have been developed to simulate the physiological responses of the body during HUT [40][41][49]. In addition, the NASA Digital Astronaut Project is currently working to establish models of bone loss due to skeletal unloading, models of renal stone formation, models of ocular changes, and changes in heart shape and stress distribution in microgravity [50].

In comparison with previous studies, our experimental design features several unique characteristics. Most cardiovascular hypo-gravity studies have been carried out in parabolic-flights or using bed rest but few of them included the exercise factor. In contrast, studies including the exercise factor were performed using suspension or lower body positive pressure and are normally focused on musculoskeletal or aerobic power results. With our approach, we are able to gain unique insights into the body's multi-system interaction when exercising under hypo-gravity conditions. Similar studies have been carried out in hyper-gravity using centrifuges [51][52][53] and our research would contribute to complete the dose-response curves obtained in hyper-gravity with G doses lower than 1g.

3.2. Potential effects of Mars gravity

The effects of Martian gravity on the musculoskeletal, cardiovascular, pulmonary and sensorimotor systems of the human body are still unknown. Some experiments carried out with mice on board the space station using the available centrifuges (JAXA ISS mice centrifuge) suggest that Martian gravity is not enough to protect against the bone loss associated with microgravity [54][55]. The only human data available has been obtained during parabolic-flights. However, new experiments are on the horizon including exposing crew members returning from space to 22.3° HUT to simulate Martian physiological adaptation [6].

Our study will contribute to fill this gap with acute musculoskeletal, pulmonary and cardiovascular responses to exercise at Mars gravitational levels, a focus that is highly relevant to future planetary surface exploration missions.

4. EXPERIMENTAL METHODS

4.1. SUBJECTS

A total of 14 healthy subjects (12 males and 2 females) capable of performing one hour of cardiovascular exercise were selected to participate in the experiment (mean \pm SD, age: 23.5 ± 3.5 years; height: 177.6 ± 8.0 cm; weight: 71.9 ± 7.8 kg). The exclusion criteria included any cardiopulmonary medical conditions, recent musculoskeletal injuries, or medication that could put subjects at risk or bias the results.

Prior to the experiment, subjects were instructed to avoid exercising and to abstain from drinking caffeine the morning prior to testing. All of them were informed about their right to withdraw from the experiment at any point and provided written informed consent to participate. The study was approved by Cornell University's Institutional Review Board for Human Participants (IRB).

4.2. ALTERED-GRAVITY EXERCISE PLATFORM SIMULATOR

The Cornell Altered-gravity Exercise Platform Simulator (CAEPS) is a platform designed and constructed at Cornell University to perform cycling ergometer exercise in multiple, simulated gravitational environments. Using head-down tilt (HDT) and head-up tilt (HUT) positions, the CAEPS can replicate known gravity-induced fluid shifts based on appropriate tilt angles. Thus, the platform is capable of providing a 6-degree head-down tilt, a 9.5-degree head-up tilt, a 22.3-degree head-up tilt, and a 90-degree upright orientation, which corresponds to microgravity, Moon, Mars, and Earth, respectively. In the reclined positions (i.e. microgravity, Moon, and Mars), subjects laid on the platform with a pillow under their head and handlebars were positioned laterally on either side, to avoid sliding down. The handlebars had five different configurations in order to be adjustable for the subject's needs. In the upright position subjects sat on a bike seat with handlebars positioned in front of them as on a standard bike.

The platform also includes a cycle ergometer device (Lode BV, Groningen, Netherlands) to study the physiological effects of different exercise parameters, such as workload intensity, cadence and duration, within each one of the simulated altered gravity

environments (see Figures 10 and 11). The cycle ergometer can be adjusted to accommodate anthropometric differences between subjects.

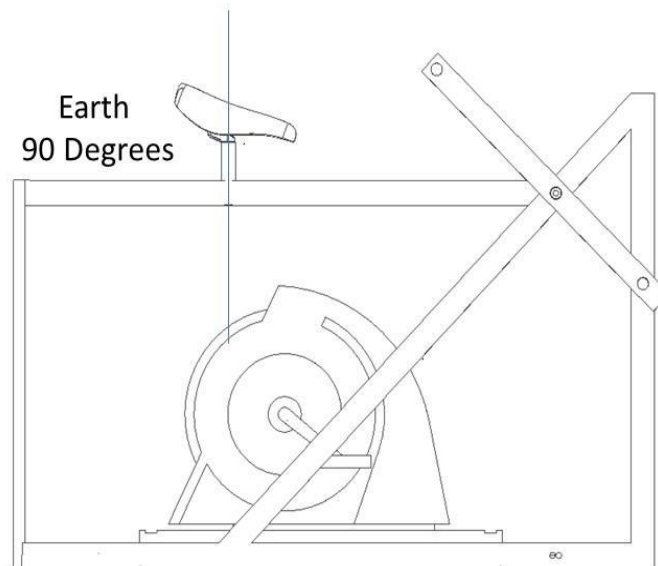


Figure 10. CMEPS device in Earth configuration.

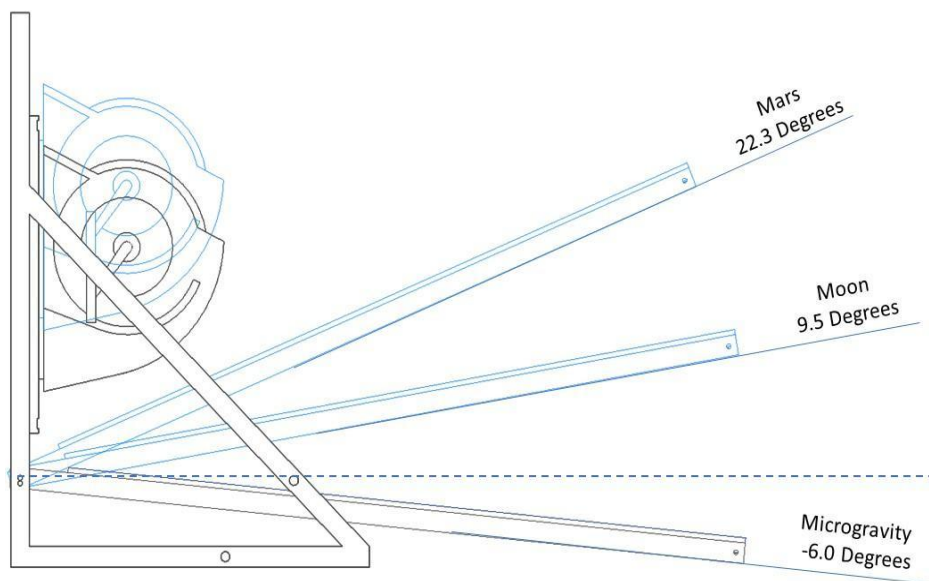


Figure 11. CMEPS device in different reclined configurations.

The Lode Ergometry Manager (LEM) software package was used to control the ergometer from a computer, allowing the operator to program different exercise protocols, visualize, save, and analyze ergometry data.

4.3. EXPERIMENTAL DESIGN

A 4x4 factorial experimental design was chosen to determine the effects of the independent variables artificial gravity level (Ag) and workload intensity (WL) on cardiopulmonary and musculoskeletal responses. The Ag levels tested were: microgravity, Moon, Mars, and Earth. The workload levels tested were 0W, 50W, 75W, and 100W. A within subjects' design was applied, such that each subject participated in every combination of workload intensity and artificial gravity.

Each subject participated in four sessions scheduled on different days within the same week. The experimental sessions were always performed in the morning approximately at the same time to avoid possible confounding circadian effects that could influence the results. During each session, subjects performed the same exercise protocol in a different gravitational environment.

Earth configuration was always tested in the first place in order to allow subjects to get familiar with the exercise protocol and testing equipment, and to obtain baseline measurements concerning subjects' fitness levels. Then, a counterbalanced design was used for the following three tests sessions (i.e. microgravity, Moon, Mars).

4.4. INSTRUMENTATION AND DATA COLLECTION

Volume of oxygen uptake (VO_2 , mL/min), volume of carbon dioxide uptake (VCO_2 , mL/min), pulmonary ventilation (V_E , L/min), tidal volume (V_T , ml) and respiratory rate (R_f , breaths/min) were recorded breath-by-breath throughout all the experiment using K4b2 portable gas analyzer (Cosmed, Srl - Italy). Prior to testing, the K4b2 main unit was warmed-up for a minimum of 45min as instructed in the system manual. The gas analyzer was calibrated before each test using a reference gas mixture (CO_2 : 5%, O_2 : 16%) (Cosmed, Srl-Italy) and the turbine was calibrated once a week with a 3000-ml syringe. The Cosmed K4b2 equipment also measured continuous heart rate data (HR) using a Polar Heart Rate monitor.

Blood Pressure measurements were taken every 2 minutes during the entire protocol using an automated blood pressure (BP) monitor, a component of the LEM system consisting of a brachial blood pressure cuff attached to the cycle ergometer. These measurements were programmed into the LEM protocol and controlled automatically by the computer. In addition to BP, other exercise parameters were measured continuously including cadence and workload.

Additionally, force-plates (Vernier Software & Technology) were mounted on the ergometer pedals. These sensors measure forces between -850 to $+3500$ N with a resolution of 1.2 N [56]. Positive force values correspond to compression force. The force-plates were zeroed before each session.

Finally, an exit survey was designed and used to collect subjective data (See Appendix 1: Questionnaires). Questions included comfort and difficulty perception of the exercise using a 10-point scale (Comfort: 1=very uncomfortable/unnatural, 10=very comfortable/natural; Strenuousness: 1=easy, 10=very strenuous), as well as the causes contributing to it. Subjects were also asked to report any muscle soreness or discomfort related with the platform orientation.

4.5. EXERCISE PROTOCOL

Each exercise session began with a 5-minute resting period in seated position in order to obtain a physiological baseline at rest. After this first period, subjects were positioned on the platform (or sat on the bike in Earth configuration) and were required to rest for seven additional minutes to capture their physiological baseline in the new gravitational configuration. Subjects then executed the exercise portion of the testing protocol, which consisted of three different workload stages of 50W, 75W and 100W. All stages were 7-minutes long and 30-second transition periods between stages were also included, as indicated in Figure 12, to avoid potential injuries. After the exercise period, an additional 7-minute resting period was included at the end of the exercise protocol to allow subjects to partially recover from the exercise. The exercise protocol was created using the Lode Ergometer Manager, Version 9.4.4 (LEM, 2013, Groningen, The Netherlands) software

package. Subjects were instructed to pedal at 1.5Hz (i.e. 90 rpm) using a metronome to avoid additional confounding factors related to pedaling cadence.

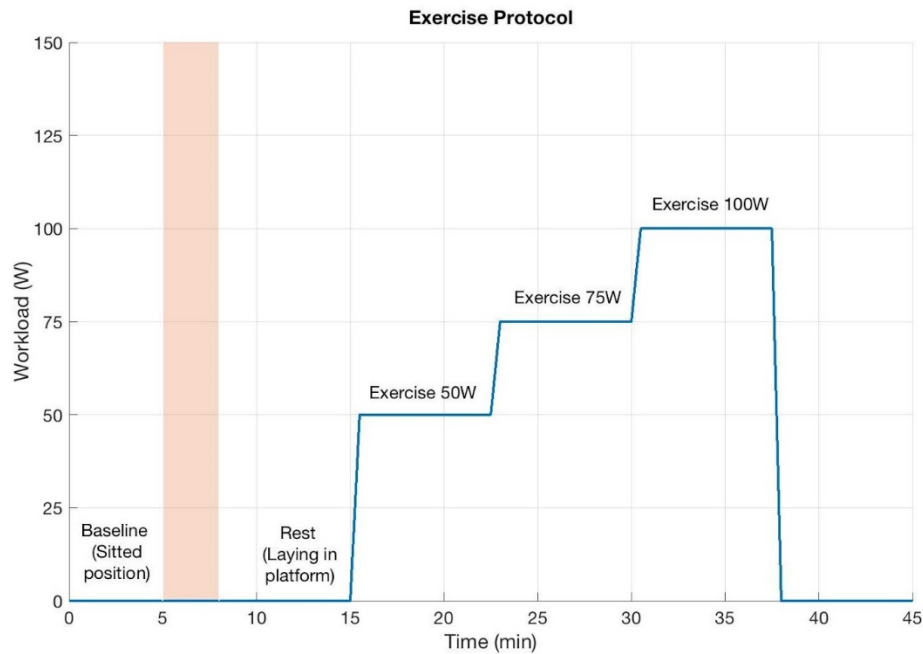


Figure 12. Exercise protocol of the experiment. Transition time represented in orange.

During the entire protocol subjects were instructed to avoid talking and making unnecessary movements that could affect data collection. Additionally, an early termination protocol was in place to ensure the safety of the subjects throughout the experiment. Termination criteria included an increase in heart rate $> 0.8 \times (220 - \text{Subject Age})$, an increase in diastolic blood pressure $> 20\text{mmHg}$ with respect to seated baseline measurements, and systolic blood pressure $> 230\text{mmHg}$.

4.6. STATISTICAL ANALYSIS

All statistical tests were performed with SPSS 25 software and the significance level was set at $\alpha = 0.05$. Breath-by-breath results were averaged over 5-seconds intervals and outliers were removed using a Hampel filter. In addition, a 5th order medium filter was applied on pulmonary data to suppress the noise of the signals.

Several statistical tests were performed to analyze the results:

- Repeated measures ANOVA was used to compare means across one or two factors (Workload level and Artificial gravity). Sphericity is an important assumption in ANOVA tests that refers to the condition where the variances of the differences between all within subject variables are equal. Greenhouse-Geisser correction was applied when our data violated this assumption [57].
- Differences between two conditions were studied using paired t-tests. The Bonferroni correction was applied to account for multiple comparisons. Assumptions related with normal distribution, homoscedasticity, and outliers were checked with SPSS before running any test.
- Finally, Friedman's test was applied to compare the results of comfort and strenuousness, since these are non-parametric variables.

5. EXPERIMENTAL RESULTS

5.1. MUSCULOSKELETAL RESULTS

Left and right foot forces were recorded continuously using Logger force plates (Vernier Software & Technology) (See figure 13). After the first resting period (seated position), the subjects lay on the platform and their feet were strapped to the pedals. An increase in foot forces can be observed at this point. Foot forces remained constant until the beginning of the exercise protocol. During the pedaling period forces oscillated between maximum and minimum values. It is important to highlight that only the forces perpendicular to the pedal surface were recorded. Tangential forces are not being considered in this study.

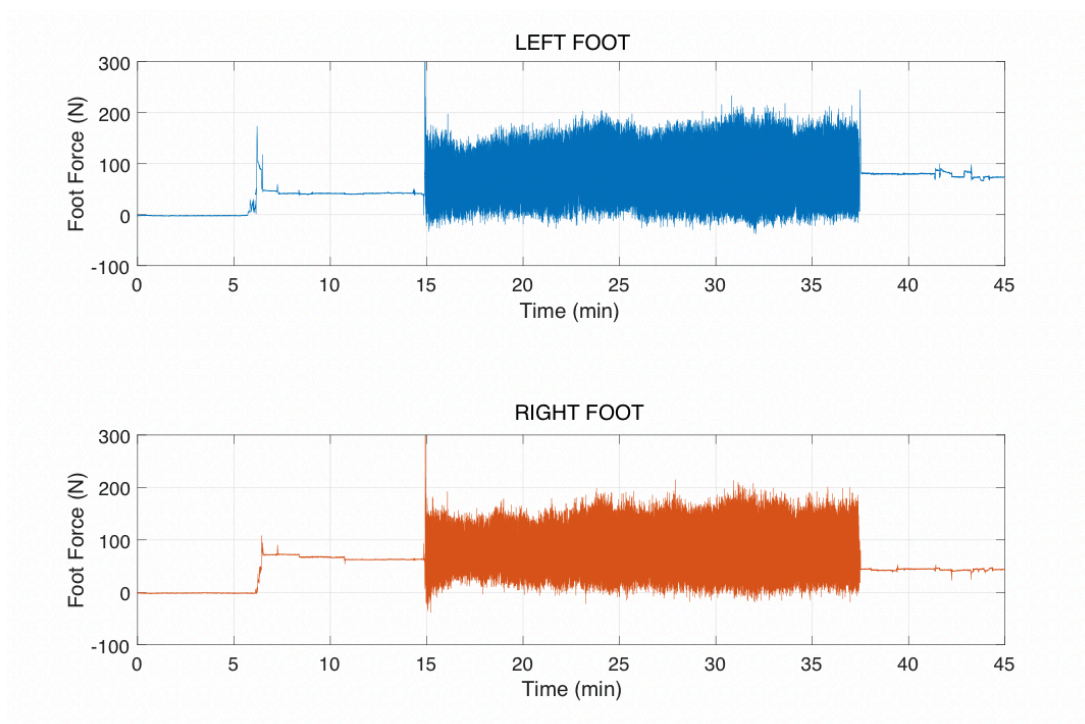


Figure 13. Pedal foot forces of one subject throughout the exercise protocol in Mars configuration (HUT 22.3 degrees)

Figure 14 illustrates the differences in the force patterns of one of the subjects with modified gravity. A shift down of the forces can be observed as gravity level decreases. Maximum forces correspond to the power phase (pedal in 0-180° position) while minimum forces occur during the recovery period (pedal in 180-360° position). Even if

maximum foot forces may seem more useful in terms of studying the generated power, this is also produced not only during the downstroke, but also during the upstroke. Thus, when minimum forces are negative (i.e. traction forces) they also contribute positively to create torque. Consequently, and in order to obtain a better understanding of these changes in the pedaling technique, both maximum and minimum forces were studied.

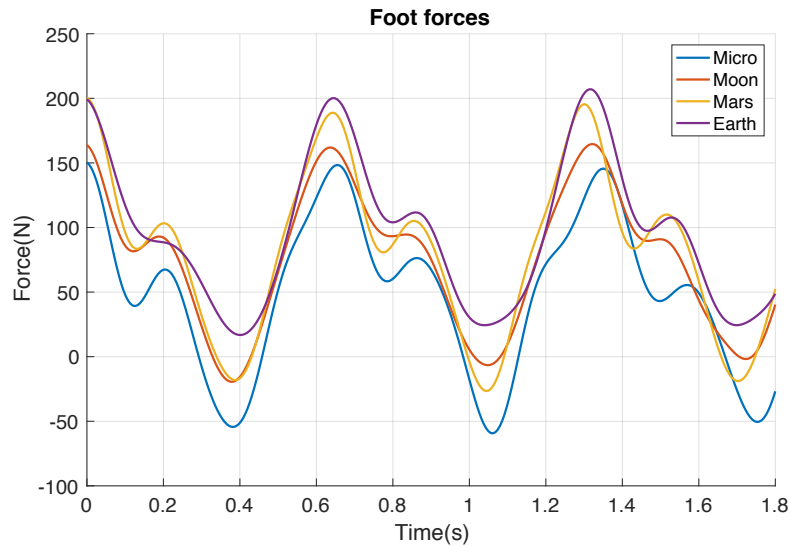


Figure 14. Foot forces pattern of one of the subjects in different Ag levels.

Mean minimum and maximum foot forces in each Ag level and work rate stage were calculated as the average of the individual maximum and minimum peak forces (transitions between stages were not included). Due to problems with the sensors in some of the tests, only 12 subjects were considered. Results are summarized in Tables 1 and 2.

Table 1. Left foot forces data presented as Mean (Standard error)

LEFT FOOT		50W	75W	100W
Micro	Max	94.94 (7.64)	98.44 (7.72)	106.46 (6.90)
	Min	-0.11 (5.78)	-7.30 (5.60)	-6.91 (6.48)
Moon	Max	125.12 (6.42)	136.54 (7.07)	144.74 (8.19)
	Min	23.75 (4.70)	18.18 (4.65)	16.88 (4.54)
Mars	Max	149.60 (9.64)	160.61 (10.66)	167.21 (10.39)
	Min	36.89 (4.70)	29.31 (6.68)	27.67 (6.67)
Earth	Max	152.44 (10.71)	169.96 (11.47)	186.96 (10.63)
	Min	49.50 (4.69)	46.67 (6.12)	53.20 (5.24)

Table 2. Right foot forces data presented as Mean (Standard error)

RIGHT FOOT		50W	75W	100W
Micro	Max	89.63 (6.71)	94.66 (6.77)	105.42 (6.59)
	Min	-1.49 (7.23)	-5.16 (8.32)	-6.42 (10.26)
Moon	Max	125.36 (5.71)	141.08 (6.14)	150.44 (7.54)
	Min	28.90 (4.72)	26.84 (5.58)	22.79 (6.72)
Mars	Max	153.07 (8.64)	162.27 (9.33)	170.19 (8.05)
	Min	41.77 (6.52)	34.88 (8.05)	31.54 (8.65)
Earth	Max	147.61 (8.24)	167.33 (8.88)	185.42 (9.01)
	Min	53.09 (6.01)	51.63 (7.20)	55.22 (7.23)

A two-factor repeated-measures ANOVA was performed to show the influence of workload intensity and artificial gravity on both maximum and minimum foot forces. Artificial gravity levels considered in the study were: Microgravity, Moon, and Mars. Earth configuration was not included due to the differences in body position from reclined to Earth tests. These differences could lead to changes in inertial forces or pedaling effectiveness that are not caused by changes in gravity but by changes in pedaling configuration and therefore confound the final results. Paired t-tests were used instead to compare Earth with reclined positions. Differences between left and right forces were also analyzed.

5.1.1. Maximum foot forces

Between subjects' effect between right and left foot forces was first analyzed and no significant differences were found ($F(1,22) = 0.008$, $p = 0.927$). Consequently, both feet were studied together. Results showed that both workload intensity ($F(1,288, 29.625) = 46.534$, $p < 5 \cdot 10^{-4}$) and artificial gravity level ($F(1.587, 36.405) = 204.135$, $p < 5 \cdot 10^{-4}$) statistically affected maximum foot forces.

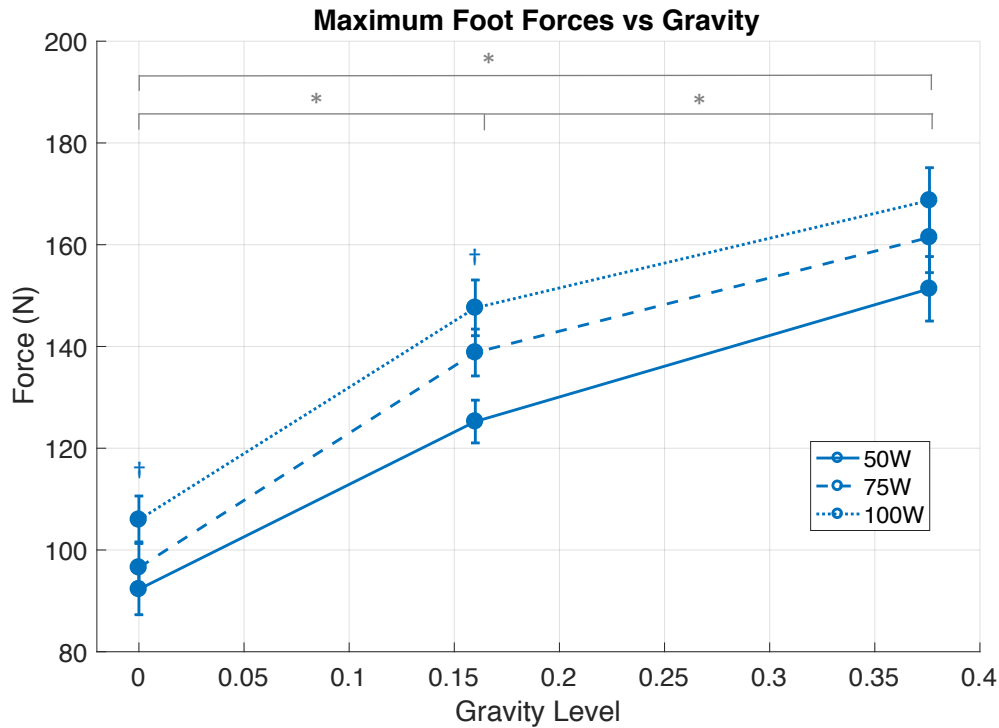


Figure 15. Maximum foot forces evolution with changes in artificial gravity level. Both right and left foot forces were considered (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction. † Significantly different from Earth using paired t-test.

Maximum foot forces were all positive and therefore compression forces. The analysis shows an increase with workload as is expected, and an increase with gravity level (See figure 15). The effect of the Ag level was also significant when analyzing pair-wise comparisons using Bonferroni corrections. Statistically significant differences were found between all the Ag conditions (i.e. Micro and Moon, Micro and Mars, Moon and Mars).

Paired t-tests revealed that maximum foot forces in the upright configuration (i.e. Earth gravitational condition) were significantly different from microgravity (50 W: $t(23) = -12.092$, $p < 5 \cdot 10^{-4}$; 75 W: $t(23) = -10.784$, $p < 5 \cdot 10^{-4}$; 100 W: $t(23) = -13.707$, $p < 5 \cdot 10^{-4}$) and Moon (50 W: $t(23) = -5.195$, $p < 5 \cdot 10^{-4}$; 75 W: $t(23) = -4.970$, $p < 5 \cdot 10^{-4}$; 100 W: $t(23) = -6.468$, $p < 5 \cdot 10^{-4}$) across all workload intensities.

5.1.2. Minimum foot forces

When studying minimum foot forces, analysis didn't show significant differences between right and left foot forces ($F(1,22) = 0.260$, $p = 0.609$). Studying both of them together, both the effect of workload intensity ($F(1.473, 32.405) = 44.506$, $p < 5 \cdot 10^{-4}$) and artificial gravity level ($F(1.407, 30.944) = 15.702$, $p < 5 \cdot 10^{-4}$) were statistically significant. Figure 16 shows how foot forces increased when increasing Ag but decrease with increased workload. In this case we obtain both positive and negative minimum forces and therefore, both compression and traction forces. Pairwise comparisons yielded statistically significant differences between all the gravity conditions.

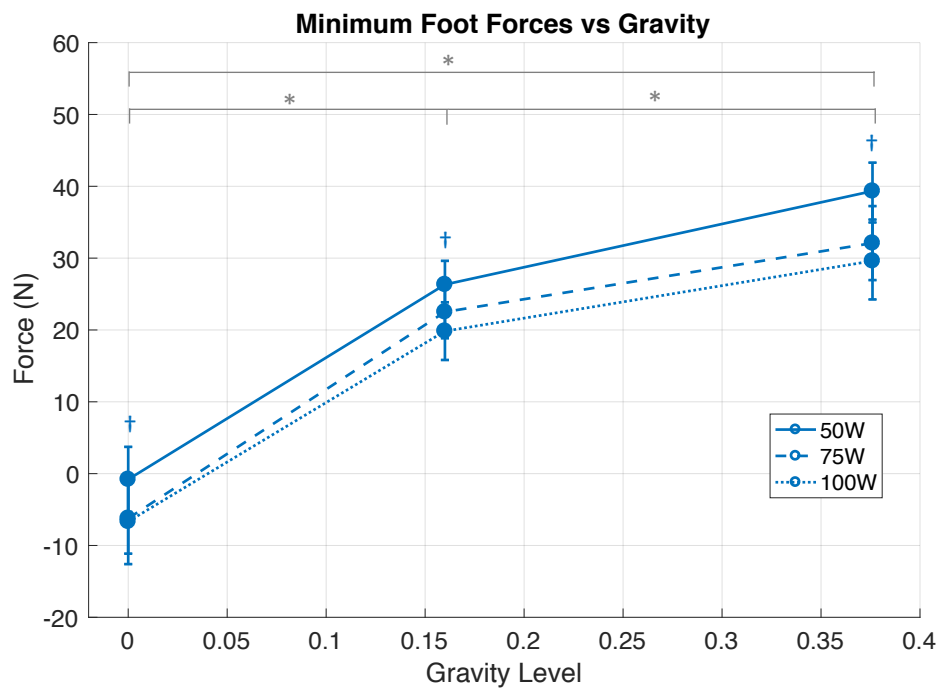


Figure 16. Minimum foot forces evolution with changes in artificial gravity level. Both right and left foot forces were considered (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction. † Significantly different from Earth using paired t-test.

Minimum foot forces generated during all of the reclined positions (i.e. Mars, Moon, microgravity) were also significantly different from the minimum foot forces generated in Earth configuration across all the workload intensities: microgravity (50 W: $t(23) = 11.107$, $p < 5 \cdot 10^{-5}$; 75 W: $t(23) = 8.652$, $p < 5 \cdot 10^{-5}$; 100 W: $t(23) = 7.508$, $p < 5 \cdot 10^{-5}$), Moon (50 W: $t(23) = 5.896$, $p < 5 \cdot 10^{-5}$; 75 W: $t(23) = 6.486$, $p < 5 \cdot 10^{-5}$; 100 W: $t(23) =$

7.680 , $p < 5 \cdot 10^{-5}$) and Mars (50 W: $t(23) = 3.204$, $p = 0.004$; 75 W: $t(23) = 4.061$, $p < 5 \cdot 10^{-5}$; 100 W: $t(23) = 5.439$, $p < 5 \cdot 10^{-5}$).

5.2. CARDIOPULMONARY RESULTS

In this section the following protocol has been used in order to decide the appropriate statistical test to study each variable.

A two-factor repeated-measures ANOVA statistical test was applied, the factors being Ag and workload intensity. Four levels were considered for each of the factors: Rest, 50W, 75W and 100W (WL); microgravity, Moon, Mars and Earth (Ag).

- a) If the interaction between the two factors was not statistically significant, these results were used for interpretation.
- b) Otherwise, 'Rest' and the other WL levels (50W, 75W and 100W) were studied separately using the following statistical tests:
 - One-factor ANOVA: Rest values were compared across the different artificial gravity levels.
 - Two-factor repeated-measures ANOVA: Both Ag and WL were considered as independent variables but, in this case, only 50W, 75W and 100W levels were used.

5.2.1. Heart Rate

Figure 17 shows the evolution of the heart rate during the experimental protocol in different gravitational conditions. After the 5-minutes baseline period, a rise in HR can be observed corresponding to the transition between seated position and the required position according to the gravity level being studied. A new steady state is attained between minutes 10 and 15. During the exercise period, heart rate increased proportionally with the workload intensity attaining a new steady state within the seven minutes of each stage. An exponential decrease can be observed at the end of the protocol when pedaling stopped.

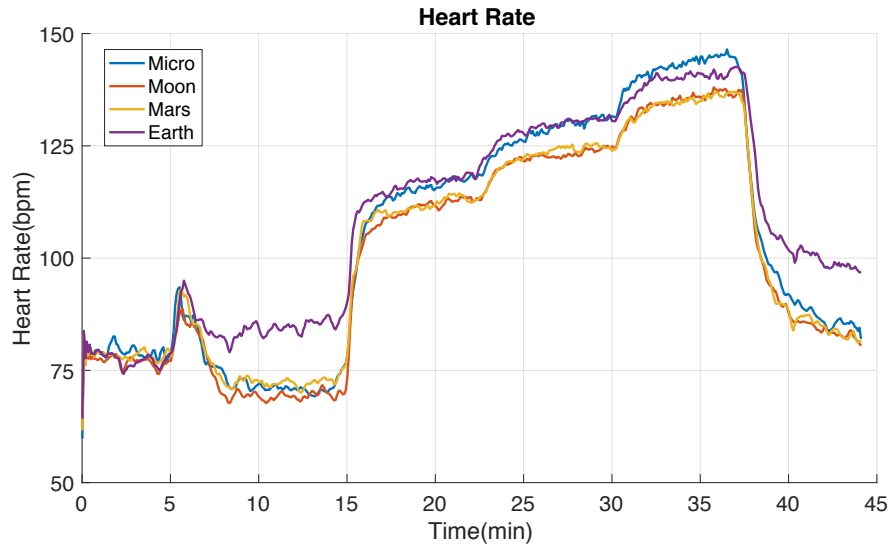


Figure 17. Averaged continuous heart rate responses from 14 subjects during the exercise protocol at different artificial gravity levels (i.e. Earth (90° HUT), Mars (22.3° HUT), Moon (9.5° HUT) and microgravity (6° HDT)).

For the statistical analysis, heart rate data was averaged within the last two minutes of each stage: Baseline, Rest, 50 W, 75W and 100W. Values are summarized in Table 3.

Table 3. Heart rate data presented as Mean (Standard error)

Heart Rate (bpm)	Baseline	0W	50W	75W	100W
Micro	78.44 (3.45)	70.61(3.22)	117.73 (3.47)	130.87 (3.77)	143.90 (3.96)
Moon	76.51 (3.47)	69.76 (3.16)	113.08 (3.31)	124.09 (3.66)	136.80 (3.75)
Mars	78.43 (3.18)	72.52 (3.81)	113.51 (4.14)	124.71 (4.16)	136.21 (4.14)
Earth	77.16 (3.83)	85.71 (3.98)	118.18 (4.44)	130.86 (4.74)	141.21 (4.55)

When analyzing the effects of gravity and workload intensity together two different tendencies were observed depending on whether the subjects were at rest or in the exercise phase. Both of them were analyzed separately to better understand body response mechanisms.

The one-factor repeated-measures ANOVA used for the rest phase shows a statistically significant effect of Ag ($F(3,39) = 24.035$, $p < 5 \cdot 10^{-4}$) on heart rate as can be seen in Figure 18. Pairwise comparisons yielded significant differences between Earth and microgravity ($p < 5 \cdot 10^{-4}$), Earth and Moon ($p < 5 \cdot 10^{-4}$) and Earth and Mars ($p < 5 \cdot 10^{-4}$).

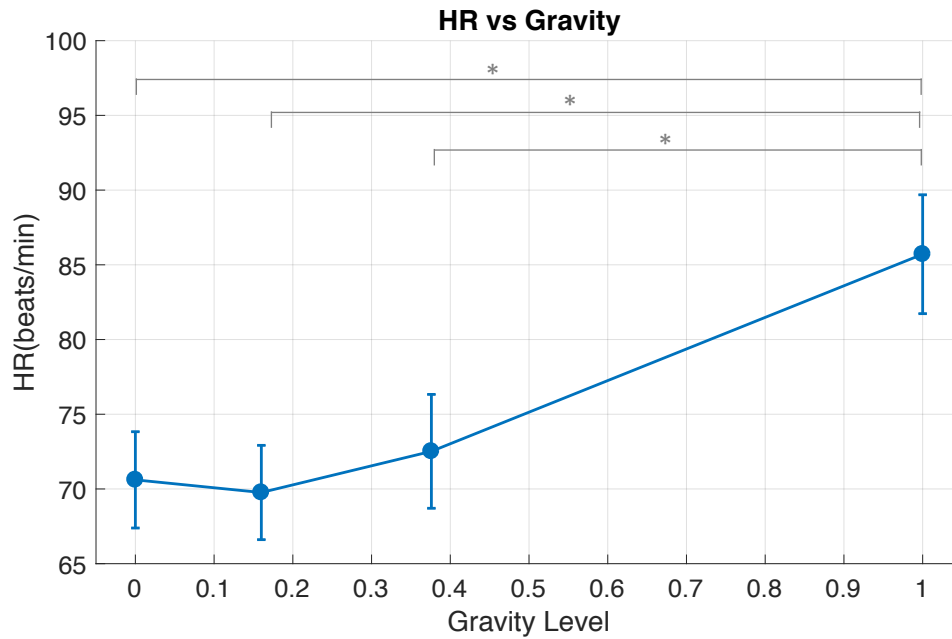


Figure 18. Heart rate in rest as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

To analyze Heart Rate responses during the exercise phase in different gravity environments we performed a two-factor repeated-measures ANOVA test. Results showed that both artificial gravity ($F(1.641, 21.337) = 6.148, p = 0.011$) and workload intensity ($F(1.228, 15.960) = 281.113, p < 5 \cdot 10^{-4}$) were statistically significantly affecting HR (See figure 19).

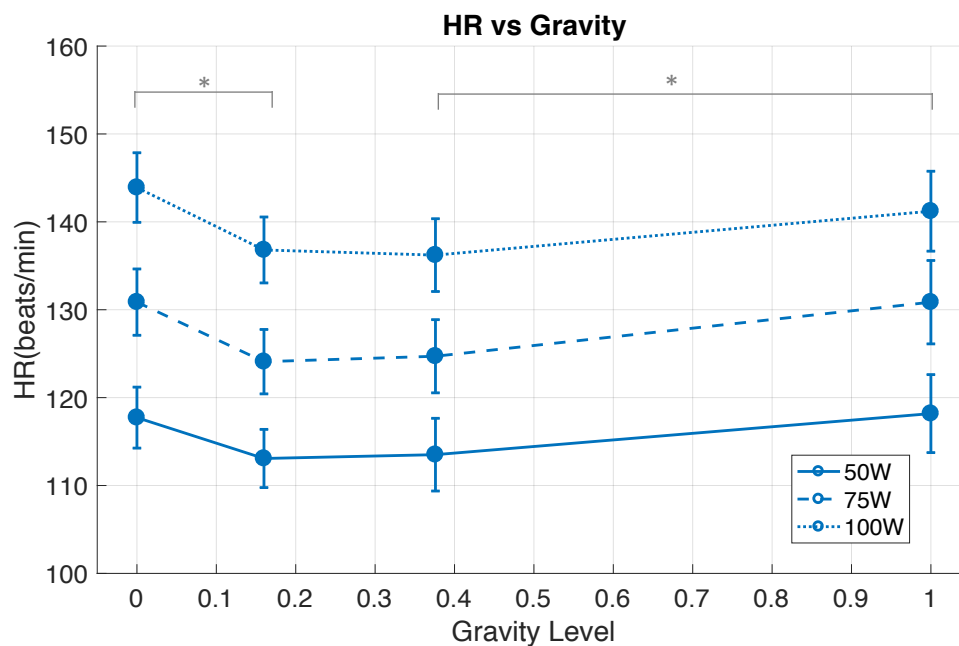


Figure 19. Heart rate responses at different workloads as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

Pairwise comparisons across gravitational conditions showed differences between microgravity and Moon ($p = 0.001$) and between Mars and Earth ($p = 0.022$).

5.2.2. Blood pressure

A total of 22 blood pressure measurements were taken during the protocol every two minutes. Mean values of each measurement for systolic and diastolic pressure under each of the four altered gravity conditions are shown in Figure 20.

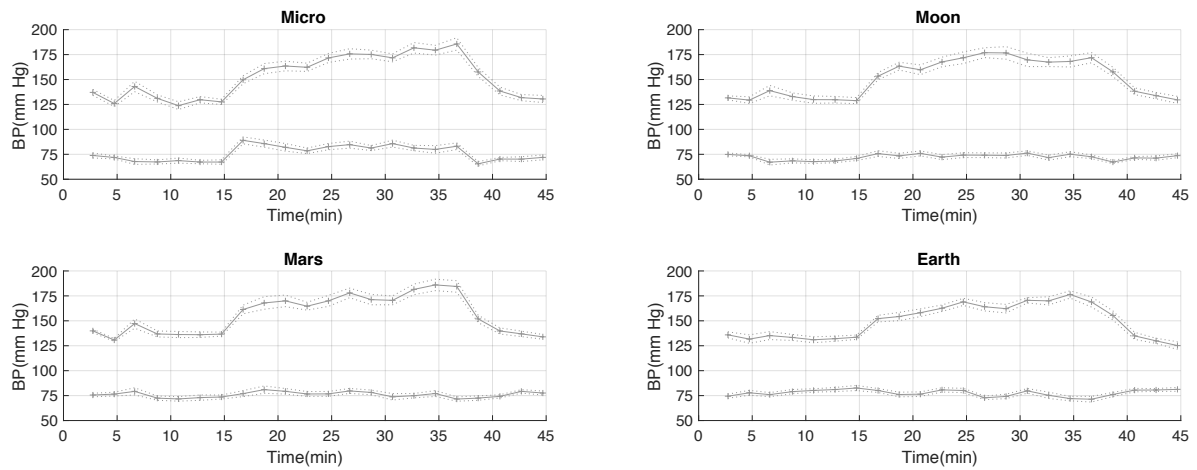


Figure 20. Systolic and diastolic blood pressure means for each CMEPS configuration (Mean \pm SE)

Diastolic blood pressure seems to stay constant during all the protocol except in microgravity conditions, where a little increase can be noticed in the exercise phase. In contrast, systolic blood pressure increases with every workload transition. A peak in SBP can also be noticed after minute 5 when the change in position occurred.

An overall blood pressure value for each workload stage was calculated as the mean of the two last measurements taken inside this period to ensure steady state has been achieved (See Table 4). However, some disarranges with the blood pressure cuff led to missing data in our set. If only one of the measurements used in each stage was missing the other one was considered for the studies. If both of them were missing Multiple Imputation techniques were applied [58].

Table 4. Time intervals of each stage and measurements used to calculate the blood pressure values in each period.

	Baseline	0W	50W	75W	100W
Measurement number	1,2	5,6	9,10	13,14	17,18
Time interval (minutes)	[1 5]	[8 15]	[15.5 22.5]	[23 30]	[30.5 37.5]

A two-way repeated-measures ANOVA was performed for both systolic and diastolic blood pressure with Ag and Workload level as independent variables. Systolic blood pressure results were significantly affected by both Ag ($F(3,39) = 3.378$, $p = 0.028$) and workload level ($F(3,39) = 175.684$, $p < 5 \cdot 10^{-4}$). Figure 21 shows SBP as a function of Ag. A peak in the response can be observed at the Mars gravity level which does not follow a homogeneous relationship. However, when applying Bonferroni corrections, pairwise comparisons showed no statistically significant differences between any of the Ag levels.

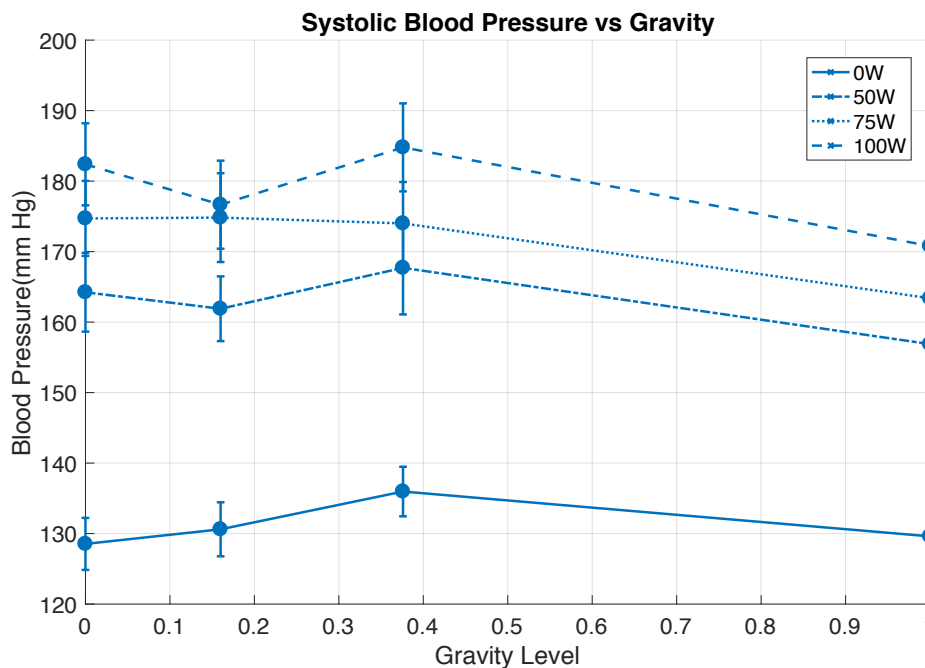


Figure 21. Systolic blood pressure results at different WL as a function of Ag. (Mean \pm SE)

Statistical analysis on diastolic BP showed a significant interaction of Ag with WL and therefore rest and exercise phases were studied separately.

Responses at rest showed a statistically significant increase of DBP with Ag ($F(3,39) = 9.879$, $p < 5 \cdot 10^{-4}$) as we can see in figure 22. However, when introducing exercise, results showed the opposite behavior (See figure 23). Diastolic blood pressure significantly decreases with gravity ($F(3,39) = 5.902$, $p = 0.02$) and is not affected by WL ($F(2,26) = 2.015$, $p = 0.157$). This behavior is quite similar to the one we observed with heart rate; the variable increases at a higher rate in lower artificial gravity levels when exercising. This is especially noticeable in microgravity conditions.

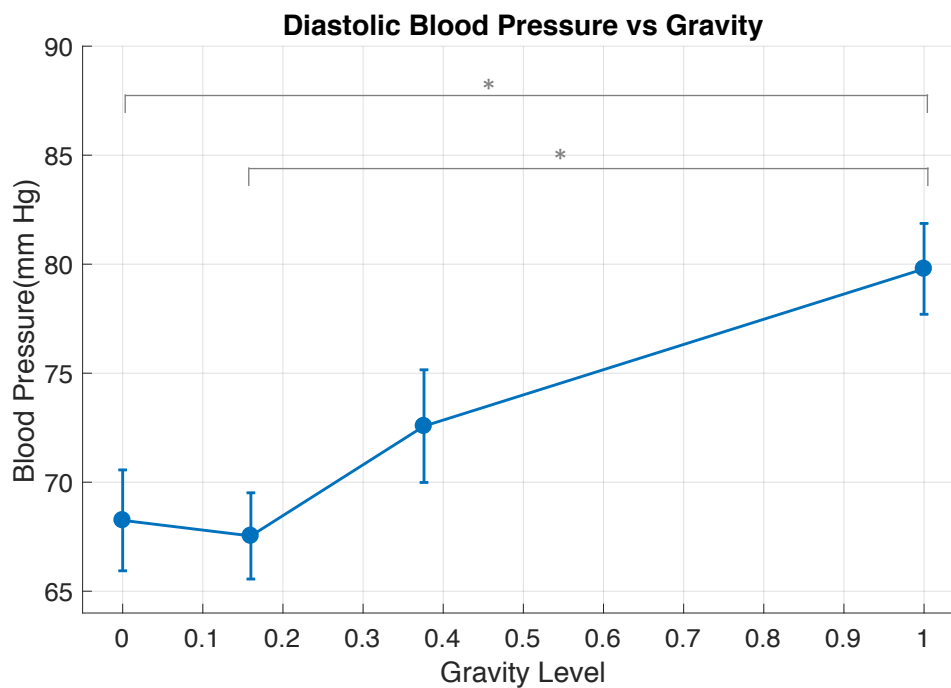


Figure 22. Diastolic blood pressure responses at rest as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

Pairwise comparison at rest using Bonferroni post-hoc corrections show significant differences between microgravity and Earth ($p = 0.011$) and between Moon and Earth ($p = 0.002$). In the exercise phase, differences were only significant between microgravity and Moon conditions ($p = 0.001$).

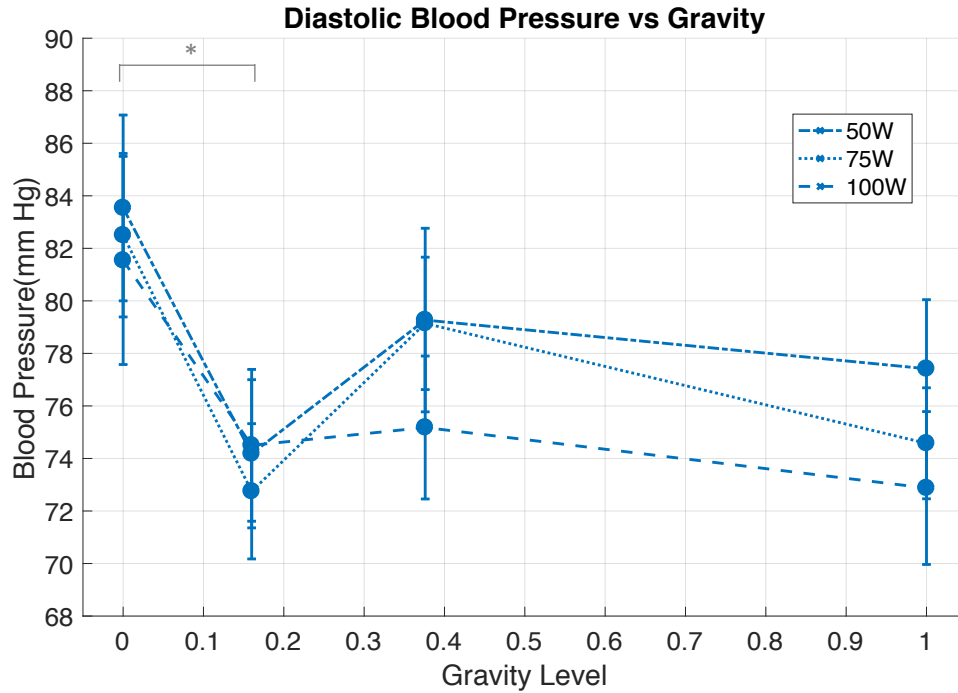


Figure 23. Diastolic blood pressure responses with exercise as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

In order to get a better picture of the effects of Ag on the cardiovascular system, mean arterial pressure (MAP) was also studied. When performing the statistical test, the interaction between the two factors (Artificial gravity level and Workload) was significant and therefore rest and exercise phases were studied independently.

Rest analysis showed that Ag effect was statistically significant ($F(3,39) = 4.960, p = 0.005$). The relationship between MAP and Ag can be observed in figure 24, where there is a noticeable rise in pressure between Moon and Mars levels. Pair-wise comparison showed statistical differences only between Moon and Earth ($p = 0.039$).

During the exercise phase both Ag level ($F(3,39) = 6.214, p = 0.001$) and WL intensity ($F(2,26) = 4.709, p = 0.018$) effects were significant. Figure 25 shows these results where higher MAP levels can be noticed at microgravity and Mars gravity levels. Bonferroni corrections showed statistically significant differences between microgravity and Moon ($p = 0.021$) and between microgravity and Earth ($p = 0.010$).

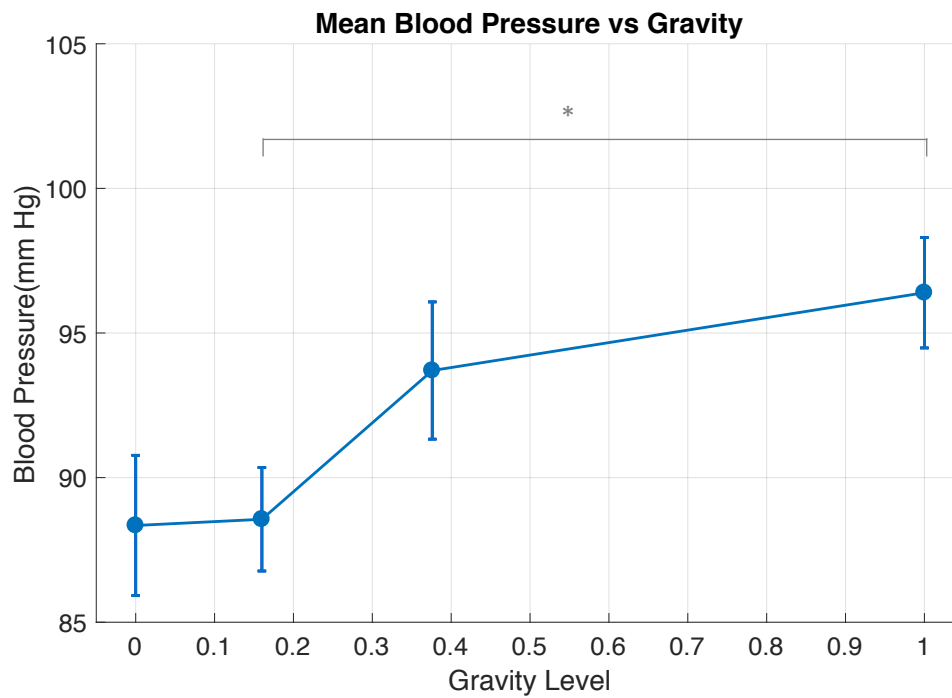


Figure 24. Mean blood pressure responses at rest as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

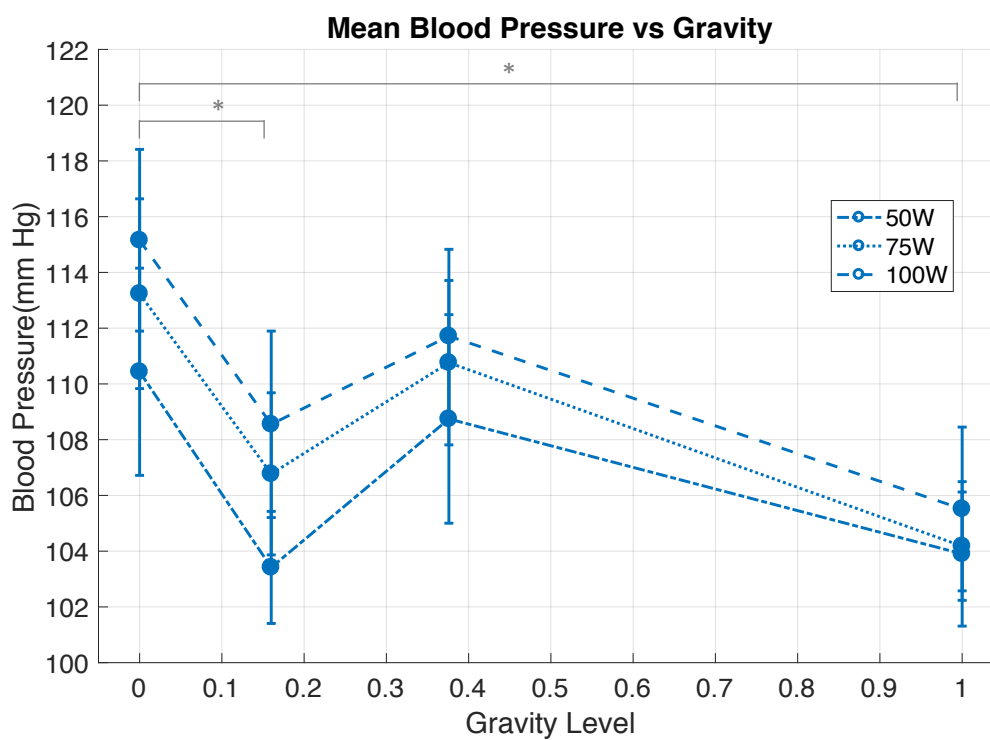


Figure 25. Mean blood pressure responses with exercise as a function of Ag (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

5.2.3. Pulmonary results

Pulmonary data was obtained with the K4b2 metabolic cart on a breath by breath basis. The results presented in this section are: Volume of oxygen uptake (VO_2), volume of carbon dioxide uptake (VCO_2), tidal volume (VT), pulmonary ventilation (VE) and respiratory rate (Rf). Continuous responses are plotted in figure 26.

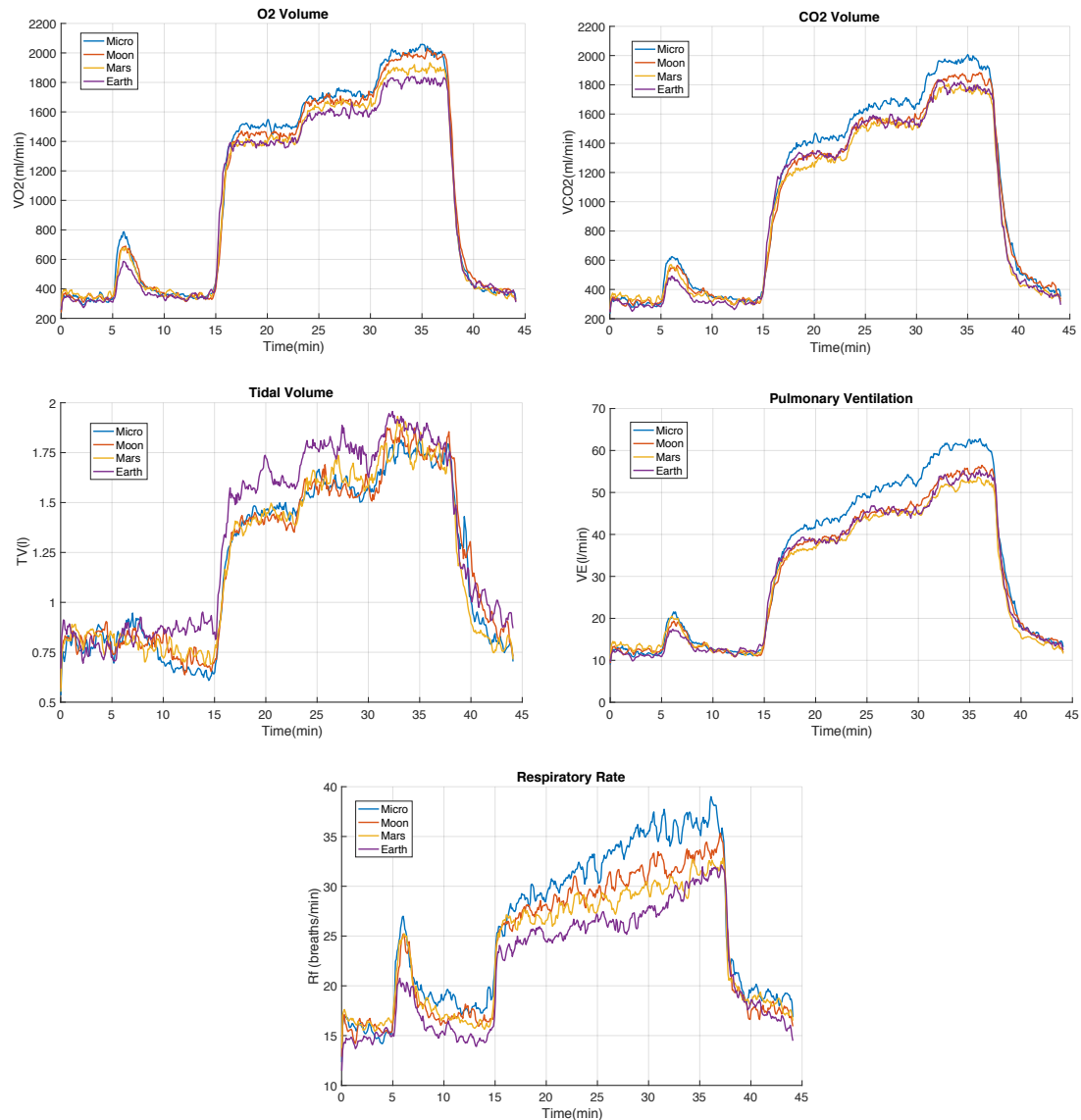


Figure 26. Averaged pulmonary continuous responses for 14 subjects in each of the four Ag levels (i.e. Earth (90° HUT), Mars (22.3° HUT), Moon (9.5° HUT) and microgravity (6° HDT)).

VO_2 , VCO_2 and V_E responses show a similar pattern along the exercise protocol. Abrupt increases can be observed in each transition between different workload levels followed

by the establishment of a new steady state in order to meet the new oxygen demand of the body. In contrast, tidal volume and respiratory rate show much more noisy curves. Tidal volume increases after every change in workload but instead of staying constant seems to have a tendency to decrease. This phenomenon is compensated by the respiratory rate, which is far from attaining a steady state and keeps increasing along the duration of every workload period. Despite the higher variability of these two variables, they seem to work closely together to maintain pulmonary ventilation ($V_E = R_f \cdot T_v$) at the adequate levels. A small increase can also be seen in all of the variables, except for tidal volume, after minute 5 due to the transition between positions.

In order to analyze the results, means for every workload level and Ag condition were calculated as the average of the last two minutes of each stage.

5.2.3.1. Volume of oxygen uptake

VO₂ mean values and standard errors are summarized in Table 5. The first statistical analysis showed an interaction between the two factors and therefore, rest and exercise responses were studied separately.

Table 5. Volume of oxygen uptake values presented as Mean (Standard error).

VO ₂ (ml/min)	Baseline	0W	50W	75W	100W
Micro	331.0 (21.1)	345.8 (14.5)	1497.1 (45.2)	1719.7 (47.3)	1973.7 (59.2)
Moon	339.2 (20.2)	350.4 (16.8)	1443.8 (43.2)	1678.9 (39.8)	1978.5 (44.0)
Mars	346.2 (25.8)	350.7 (20.2)	1408.3 (38.9)	1645.6 (38.4)	1879.6 (32.5)
Earth	319.6 (17.2)	354.1 (18.5)	1389.1 (25.2)	1597.6 (29.5)	1802.8 (36.2)

Rest results showed no significant changes in VO₂ with different artificial gravity levels ($F(3, 39) = 0.095$, $p = 0.962$) suggesting a similar metabolic rate at rest despite the position.

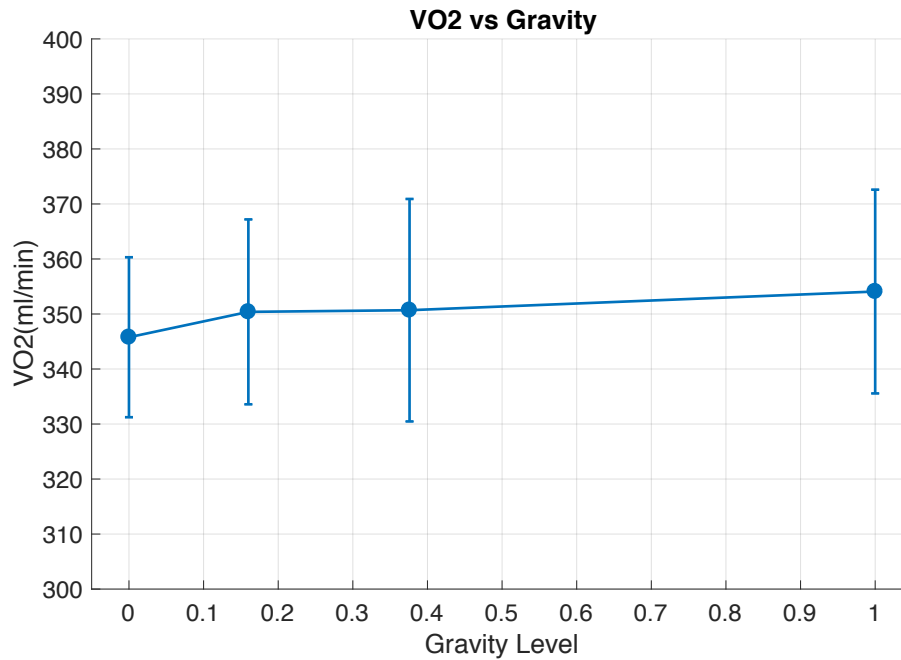


Figure 27. VO2 responses at rest as a function of Ag. (Mean \pm SE)

However, when analyzing the exercise results both factors were significantly affecting VO₂ (Ag: $F(3, 39) = 5.838$, $p = 0.002$; WL: $F(1.169, 15.195) = 509.585$, $p < 5 \cdot 10^{-4}$). However, post-hoc testing using the Bonferroni correction showed no statistically significant differences between positions. These results could suggest an increase of the oxygen demand of our body when exercising in lower gravity levels (See figure 28).

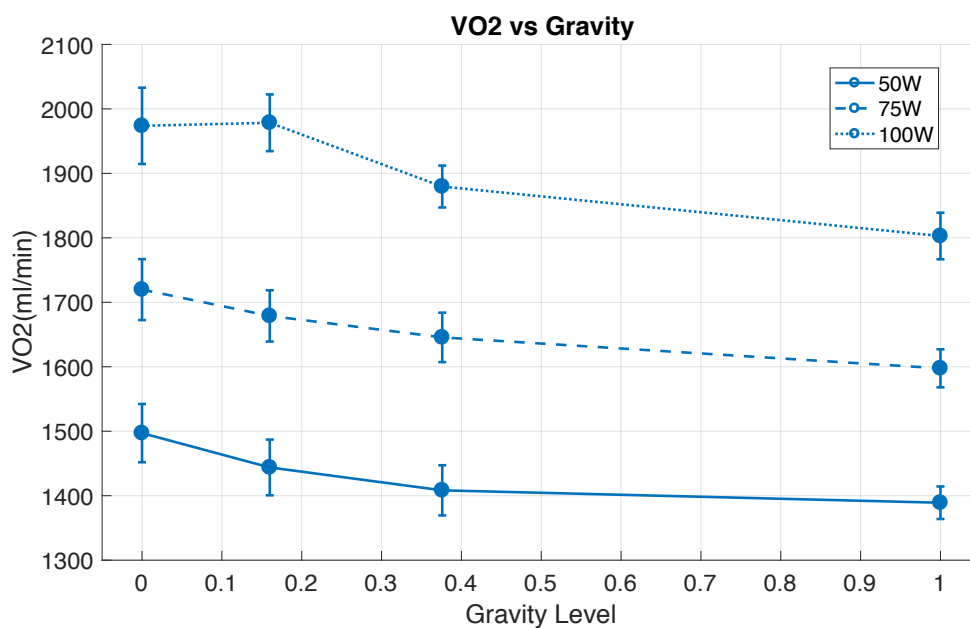


Figure 28. Oxygen uptake responses to Ag at different Workload levels (Mean \pm SE).

5.2.3.2. Volume of carbon dioxide intake

Table 6 includes the mean and standard error values for each CAEPS position in every stage of the exercise protocol. As in VO_2 results, interaction between factors was significant and responses to rest and exercise were studied independently.

Table 6. Volume of carbon dioxide uptake values presented as Mean (Standard error).

VCO_2 (ml/min)	Baseline	0W	50W	75W	100W
Micro	297.8 (18.5)	323.6 (14.7)	1433.7 (41.5)	1673.2 (45.1)	1910.9 (53.8)
Moon	310.8 (26.9)	324.9 (16.5)	1319.2 (40.7)	1550.1 (35.5)	1836.0 (44.0)
Mars	326.5 (29.5)	318.8 (20.5)	1294.6 (38.0)	1523.7 (33.3)	1740.3 (32.8)
Earth	288.6 (18.8)	314.1 (20.4)	1315.3 (27.1)	1548.5 (30.3)	1762.8 (44.0)

Results were similar to the ones of volume of oxygen uptake. VCO_2 was not affected by the different positions when at rest ($F(3, 39) = 0.177$, $p = 0.911$).

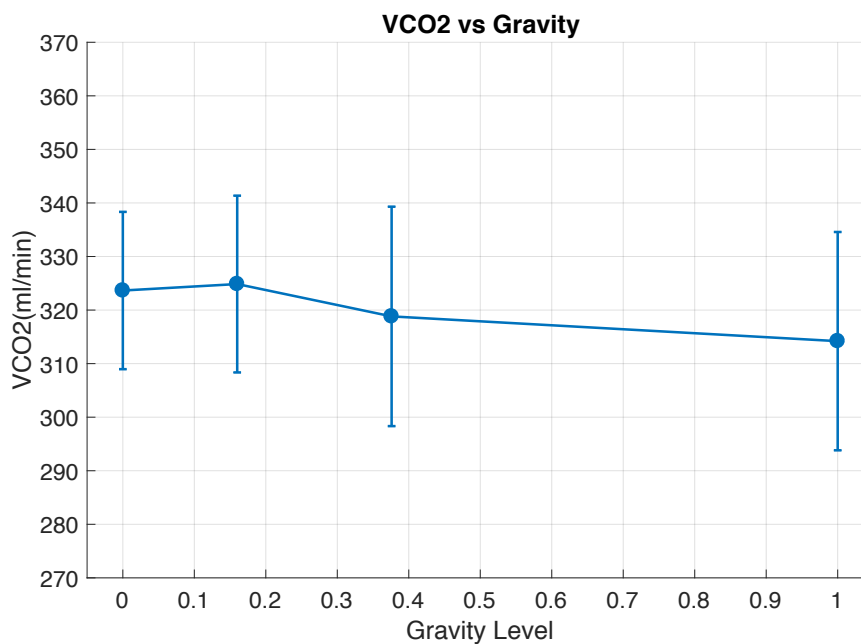


Figure 29. VCO_2 responses at rest as a function of Ag. (Mean \pm SE)

The two-factor repeated-measures ANOVA revealed a significant influence of both Ag ($F(3, 39) = 6.108$, $p = 0.002$) and WL ($(1.402, 18.221) = 364.158$, $p < 5 \cdot 10^{-4}$) in volume of carbon dioxide exhaled during the exercise period. Pairwise comparisons using

Bonferroni correction showed differences between microgravity and Moon ($p = 0.024$) and between microgravity and Mars ($p = 0.024$).

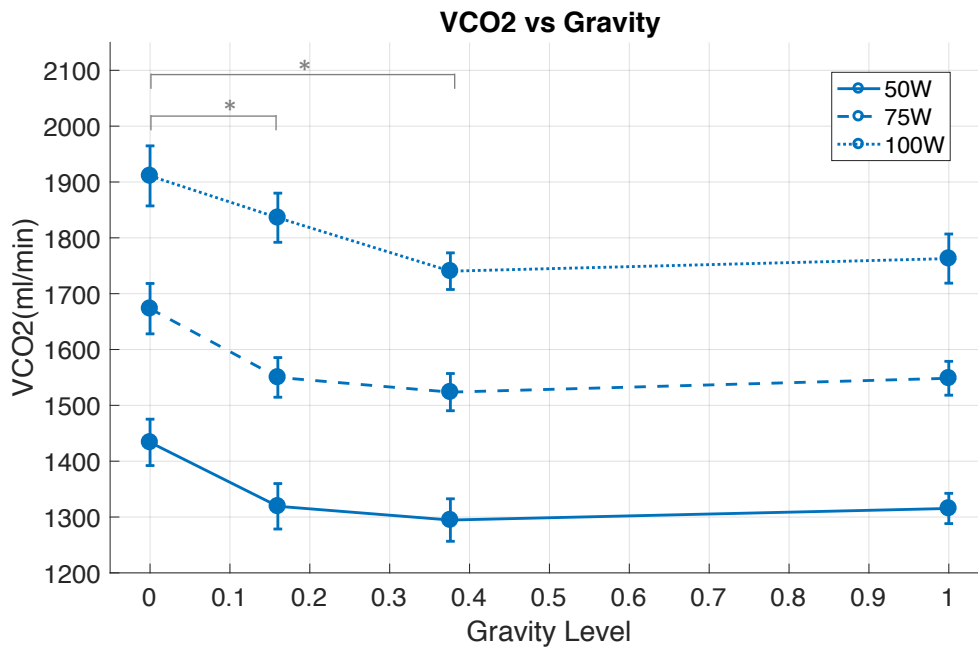


Figure 30. Carbon dioxide exhaled responses to Ag at different Workload levels (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction

5.2.3.3. Tidal Volume

Table 7 summarizes tidal volume responses. A first ANOVA statistical test showed no significant interaction between Ag and WL. Artificial gravity had a positive and statistically significant effect on VT ($F(3, 39) = 8.094$, $p < 5 \cdot 10^{-4}$). An increase in workload level also increased significantly VT ($F(1.365, 17.742) = 94.344$, $p < 5 \cdot 10^{-4}$).

Table 7. Tidal volume values presented as Mean (Standard error).

TV (l)	Baseline	0W	50W	75W	100W
Micro	0.82 (0.12)	0,64 (0.03)	1.46 (0.09)	1,55 (0.10)	1,72 (0.11)
Moon	0.83 (0.12)	0.71 (0.04)	1.41 (0.09)	1.56 (0.09)	1.74 (0.11)
Mars	0.82 (0.08)	0.74 (0.06)	1.44 (0.09)	1.61 (0.11)	1.73 (0.11)
Earth	0.77 (0.07)	0.89 (0.11)	1.60 (0.09)	1.75 (0.09)	1.82 (0.12)

Pairwise comparisons were also studied, and statistically significant differences were found between the following pairs of conditions: Microgravity with Earth ($p = 0.031$),

Moon with Earth ($p = 0.001$) and Mars with Earth ($p = 0.004$). In figure 31 we can observe the slight general increase of the tidal volume when increasing gravity.

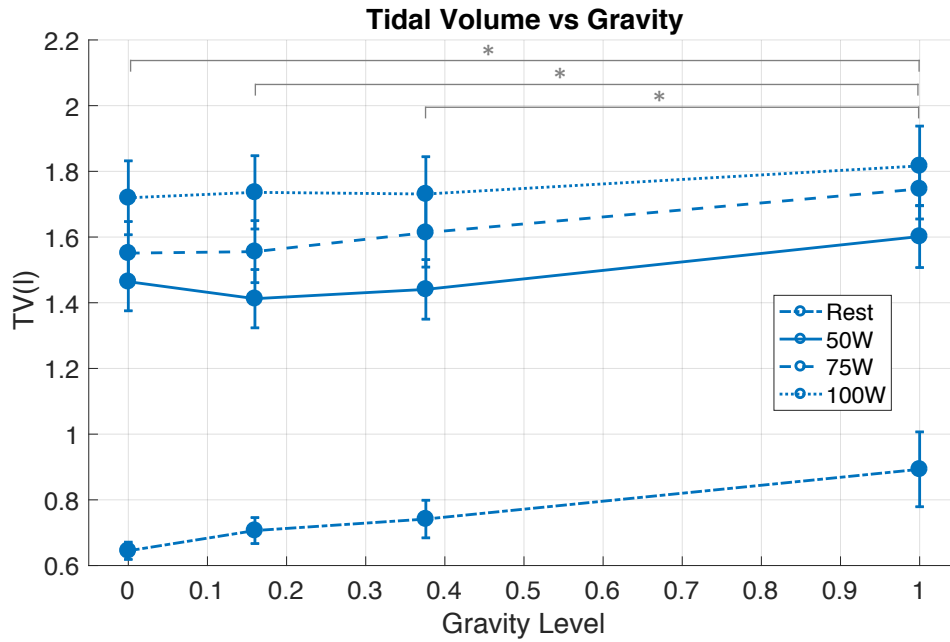


Figure 31. Tidal volume responses to Ag at different Workload levels (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

5.2.3.4. Pulmonary ventilation

The means and standard errors calculated for each tilted position and protocol stage are shown in Table 8. The first two-factor ANOVA that took into account all the workload levels showed a significant interaction between the two factors and therefore rest was studied independently.

Table 8. Pulmonary ventilation values presented as Mean (Standard error).

VE (l/min)	Baseline	0W	50W	75W	100W
Micro	11.49 (0.52)	11.57 (0.41)	43.42 (1.33)	52.71 (1.63)	60.16 (2.13)
Moon	12.28 (0.82)	11.70 (0.46)	39.35 (0.98)	46.39 (0.73)	55.01 (1.34)
Mars	12.89 (0.97)	11.80 (0.72)	38.53 (1.05)	45.13 (0.99)	52.11 (1.26)
Earth	11.11 (0.51)	12.48 (0.69)	38.59 (0.78)	45.37 (1.05)	54.00 (2.29)

The comparison between artificial gravity level at rest showed pulmonary ventilation is not affected by this factor ($F(1.817, 23.616) = 0.864$, $p = 0.425$) as we can see in figure

32. However, when studying responses with exercise Ag became statistically significant ($F(3, 39) = 10.514, p < 5 \cdot 10^{-4}$) along with WL ($F(2, 26) = 220.372, p < 5 \cdot 10^{-4}$).

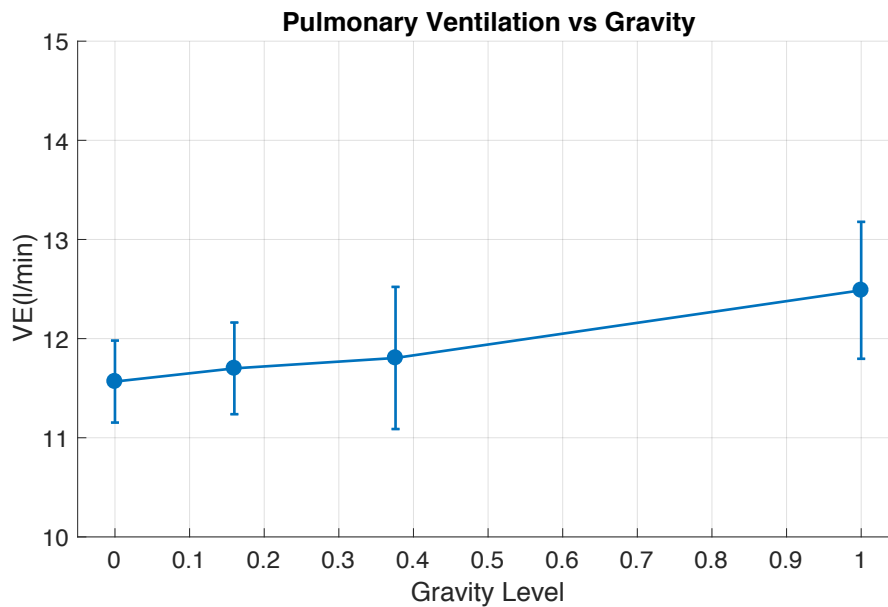


Figure 32. Pulmonary ventilation at rest as a function of Ag (Mean ± SE).

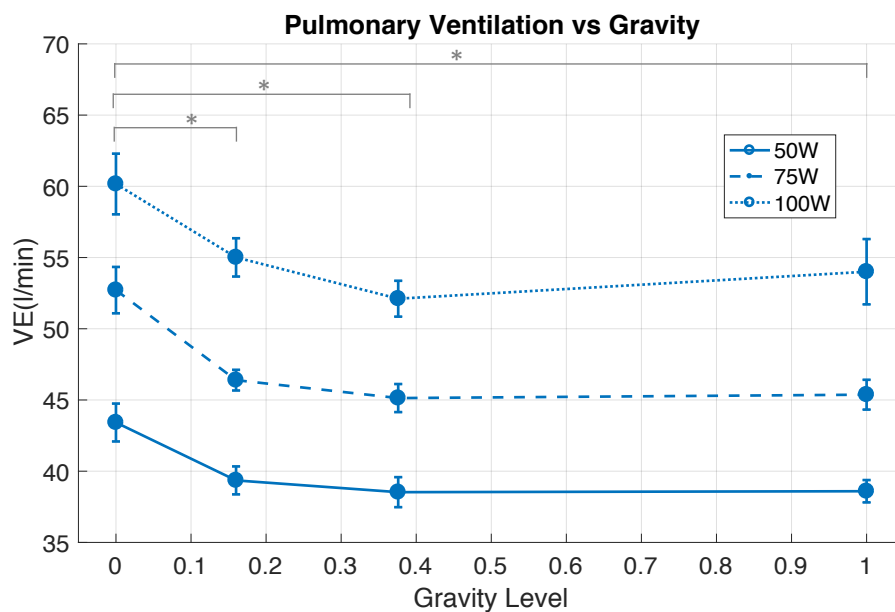


Figure 33. Pulmonary ventilation responses to Ag at different Workload levels (Mean ± SE). * Significantly different using pairwise comparisons with Bonferroni correction.

In Figure 33 we can observe how pulmonary ventilation decreases when going from 0g to 0.4g followed by a more stable response until 1g. Bonferroni pairwise comparisons

showed statistically significant differences between microgravity and Moon ($p = 0.003$), Mars ($p = 0.005$) and Earth ($p = 0.028$).

5.2.3.5. Ventilation rate

Finally, Table 9 shows the average results and standard errors for the ventilation rate data. No significant interaction between Ag and WL was found with the ANOVA statistical test. Results show that ventilation rate decreased with Ag and increased with WL, both of which were statistically significant (Ag: $F(3,39) = 17.585$ $p < 5 \cdot 10^{-4}$; WL: $F(1.428, 18.564) = 76.589$ $p < 5 \cdot 10^{-4}$).

Table 9. Ventilation rate values presented as Mean (Standard error).

VE (l/min)	Baseline	0W	50W	75W	100W
Micro	14.99 (0.99)	18.00 (0.82)	30.44 (1.84)	35.20 (2.30)	36.31 (2.44)
Moon	15.61 (0.92)	16.67 (0.76)	28.72 (1.84)	31.23 (1.92)	33.73 (2.57)
Mars	16.18 (0.90)	16.05 (0.98)	27.42 (1.71)	29.53 (1.98)	31.87 (2.27)
Earth	15.15 (0.92)	14.66 (0.88)	25.11 (1.54)	26.92 (1.41)	31.33 (2.37)

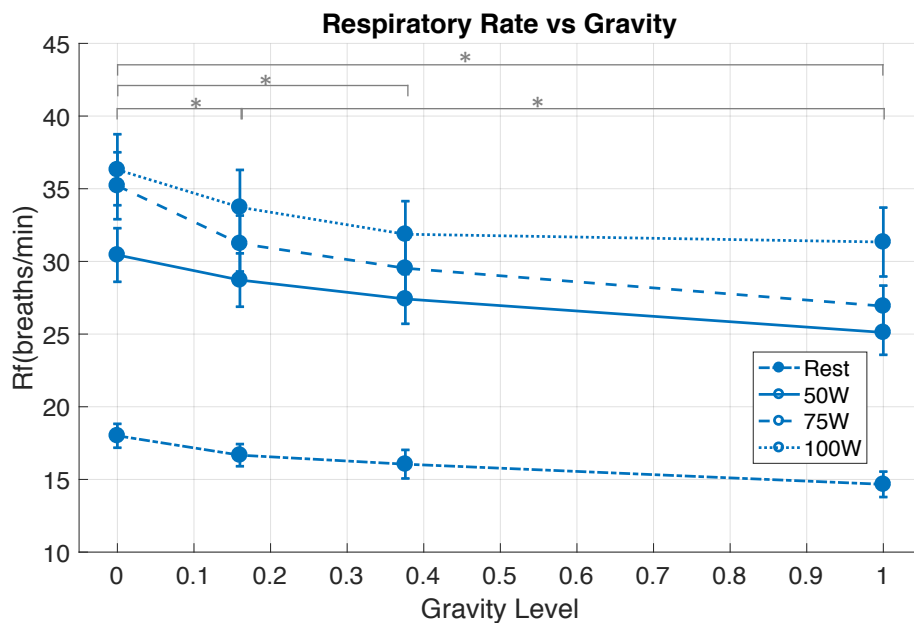


Figure 34. Respiratory rate responses to Ag at different Workload levels (Mean \pm SE). * Significantly different using pairwise comparisons with Bonferroni correction.

When comparing the different positions one by one using Bonferroni correction, statistically significant differences were found between microgravity and Moon ($p = 0.008$), Mars ($p = 0.004$) and Earth ($p < 5 \cdot 10^{-4}$) and between Moon and Earth ($p = 0.024$).

5.3. COMFORT AND STRENUOUSNESS RESULTS

After each session subjects were asked to fill out an exit survey to obtain a measurement of their perception of the protocol. Comfort and difficulty of the exercise was reported using a 10-point Likert-type scale (Comfort: 1=very uncomfortable/unnatural, 10=very comfortable/natural; Strenuousness: 1=easy, 10=very strenuous). A Friedman statistical test was performed using the data of the 14 subjects to determine the influence of artificial gravity in these variables. Both of them showed statistically significant differences with Ag (C: $\chi^2(3) = 23.59$, $p < 5 \cdot 10^{-4}$; S: $\chi^2(3) = 27.51$, $p < 5 \cdot 10^{-4}$).

Table 10. Exit survey results presented as Mean (Standard error)

	COMFORT	STRENUOUSNESS
Micro	4,4 (2,0)	6,1 (2,1)
Moon	5,9 (1,5)	4,9 (2,0)
Mars	6,7 (2,2)	4,2 (1,6)
Earth	7,4 (2,0)	3,4 (1,7)

Results show an apparent increase of the comfort level with increased Ag. As expected, Earth was reported as the most natural position with little discomfort issues, mainly related with the bike saddle. In the reclined configurations, the main causes of discomfort were pressure in the lower back and use of handlebars to avoid sliding. Some cases of numbness in the feet were also reported in the Micro position probably due to the upside-down tilt of the body.

Strenuousness data shows that microgravity was the most challenging position. The perception of the difficulty of the exercise was reduced with increasing Ag. Subjects were also asked to choose the main cause of strain between workload level, protocol duration or both. Workload intensity was selected in the majority of the cases. Other factors such as cycling frequency or discomfort due to position were also mentioned.

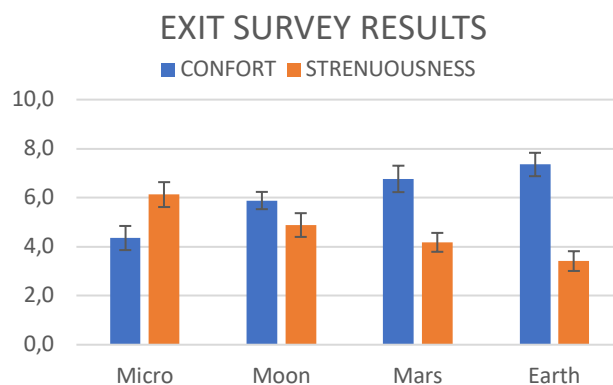


Figure 35. Comfort and strenuousness results for each Ag. Results presented as mean and standard error.

5.4. RESULTS SUMMARY

The following table summarizes the main results presented in this section. On the big picture, an increased activity of all the body's systems studied can be observed when increasing the exercise workload. In contrast, responses to the artificial gravity exposure are less clear as we will discuss in the following section.

Table 11. General responses of musculoskeletal, cardiovascular, pulmonary and physiological variables.

Physiological variable	Increased Ag level		Increased Workload level
MAXIMUM FF	Increases		Increases
MINIMUM FF	Increases		Decreases
HR	Rest	Increases	Increases
	Exercise	Non-homogeneous	
SYSTOLIC BP	Non-homogeneous		Increases
	Rest	Increases	
DIASTOLIC BP	Exercise	Decreases	Constant
VO2	Rest	Constant	Increases
	Exercise	Decreases	
VCO2	Rest	Constant	Increases
	Exercise	Decreases	
TV	Increases		Increases
VE	Rest	Constant	Increases
	Exercise	Decreases	
RF	Decreases		Increases
COMFORT	Increases		-
STRENUOUSNESS	Decreases		-

6. DISCUSSION

6.1. MUSCULOSKELETAL SYSTEM

From the skeletal point of view, some theories propose that skeletal remodeling adapts to the daily mechanical loading on our bones [59]. A magnitude of this skeletal loading during cycling can be obtained by measuring maximum peak forces as a percentage of the body weight (Table 12). According to our results these forces increased with both gravity and workload as can be seen in figure 36.

Previous studies performed onboard the ISS using the CEVIS bike measured foot forces between 7.0 and 19.0% of the body weight using workloads ranging from 75 to 210W [60]. These results are in concordance with our results in microgravity where we obtained peak forces between 12.7 and 14.6% BW. Other studies compared Earth with microgravity cycling loadings, obtaining a 20%BW and a 10%BW respectively [61]. Although these results are slightly lower compared with the ones obtained at CAEPS, differences in pedaling rate or workload level can produce changes in foot forces, being this a possible reason for these differences. Foot forces at partial-gravity levels such as the martian or the lunar ones haven't been studied previously and it is interesting to note that, in our experiment, no statistically significant differences were found between Mars and Earth conditions. Investigating this similarity could be key prescribing exercise protocols during future planetary surfaces' missions.

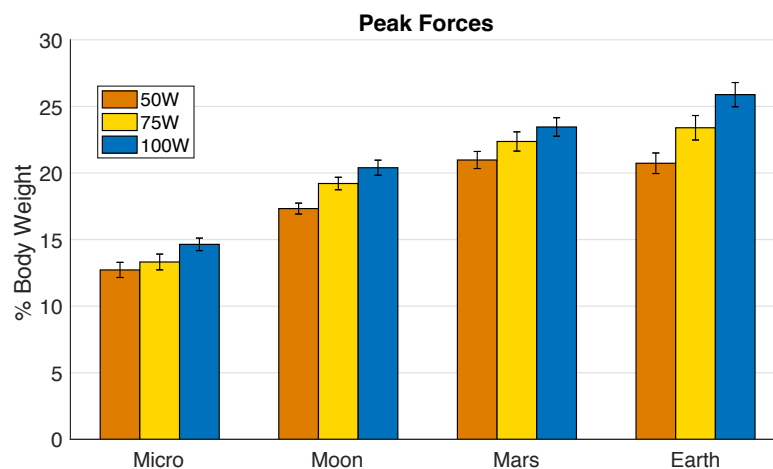


Figure 36. Peak maximum foot forces at different gravity levels and workloads. (Mean \pm SE)

Table 12. Peak maximum foot forces values as a percentage of the body weight presented as Mean (Standard error).

	50W	75W	100W
Micro	12.7 (0.6)	13.3 (0.6)	14.6 (0.5)
Moon	17.3 (0.4)	19.2 (0.5)	20.4 (0.6)
Mars	21.0 (0.6)	22.4 (0.7)	23.5 (0.7)
Earth	20.7 (0.8)	23.4 (0.9)	25.9 (0.9)

When studying the exercise impact on the muscular system both maximum and minimum forces are interesting to study. Higher workload level induced higher maximum foot forces, these representing the compression forces during the downstroke. This increase leads to a greater activation of the gluteus and quadriceps. Minimum foot forces decreased with workload, suggesting a greater contribution of traction forces at higher WL levels (Although net positive contribution is only seen in microgravity configuration). Lower negative forces lead to a higher power generation during the upstroke, increasing the activation of hamstrings and calf muscles.

Pedaling patterns were also modified across the four different gravity levels. Higher maximum and minimum foot forces are seen at greater Ag. This phenomenon can be explained by the increased weight contribution in the force's vector when increasing gravity. Interestingly, traction forces become positive in microgravity during the recovery phase. Although using pull up during the upstroke may seem to improve pedaling efficiency, some studies have shown that there is a higher metabolic cost when flexing the leg during the upstroke than when extending the leg during the down stroke [62]. According to this assumption, cycling at lower gravity levels would be associated with a higher metabolic cost and therefore, higher VO₂ consumption.

It is also important to highlight again the similarities in foot forces magnitudes between the Mars and Earth configurations (See figure 37). This result suggests similar muscle activation on both planetary surfaces. According to this foot forces' study, no artificial gravity would be needed when cycling on Mars to maintain the same Earth's musculoskeletal conditions.

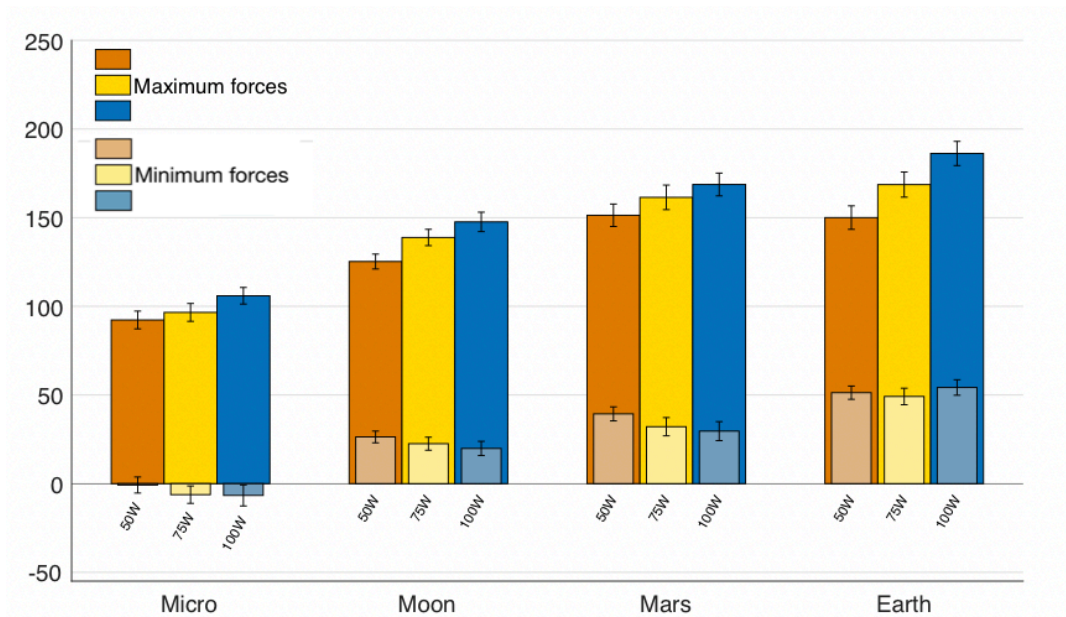


Figure 37. Comparison between maximum and minimum foot forces changes at different workload levels and Ag. (Mean \pm SE)

6.2. CARDIOVASCULAR SYSTEM

Cardiovascular responses at rest give us an idea of the isolated effect of fluid shift (i.e. with no exercise) in the CV system. Both Heart Rate and Blood Pressure increased with Ag levels at rest. As we can see in figure 38, this can be explained by the actuation of the baroreflex system. Fluid shift induces an increase in MAP triggering the baroreflex system. The aortic arch and carotid sinus receptors send inhibitory responses to the sympathetic nervous system (SNS) while the activity of the parasympathetic nervous system (PNS) is increased. The vagal nervous actuation reacts then decreasing heart rate with increased fluid shift (i.e. when decreasing the tilt angle). Mean arterial pressure results show a drop at Ag levels lower than Mars. When observing systolic and diastolic blood pressure separately we can observe that SBP is maintained across the different gravity levels while DBP falls with increasing fluid shift, leading this in the decrease of MAP. An overcompensation of the control systems seems to be happening here.

Heart rate and blood pressure responses to exercise can be explained using the diagram in Figure 39. The body responds to exercise by sending excitatory responses to the SNS as well as increasing the musculoskeletal and respiratory pumps. The SNS immediately increases heart rate (In concordance with our results). Increased activity of the pumps increases venous return which, together with the increased CO and peripheral resistance

caused by the SNS, leads to an increase in MAP. These results can also be observed in our data, with a more noticeable increase of the systolic over the diastolic blood pressure. These responses are also in concordance with the ones described by Laughlin in its review about cardiovascular responses to exercise [63].

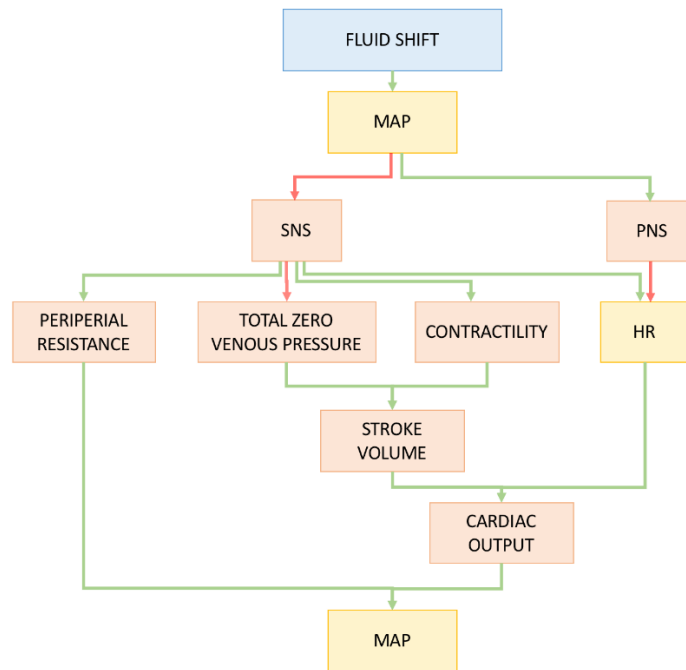


Figure 38. Block diagram showing the cardiovascular effects of the fluid shift. Green lines represent a direct relationship while red ones represent an inverse relationship. Variables measured in our study are represented in yellow.

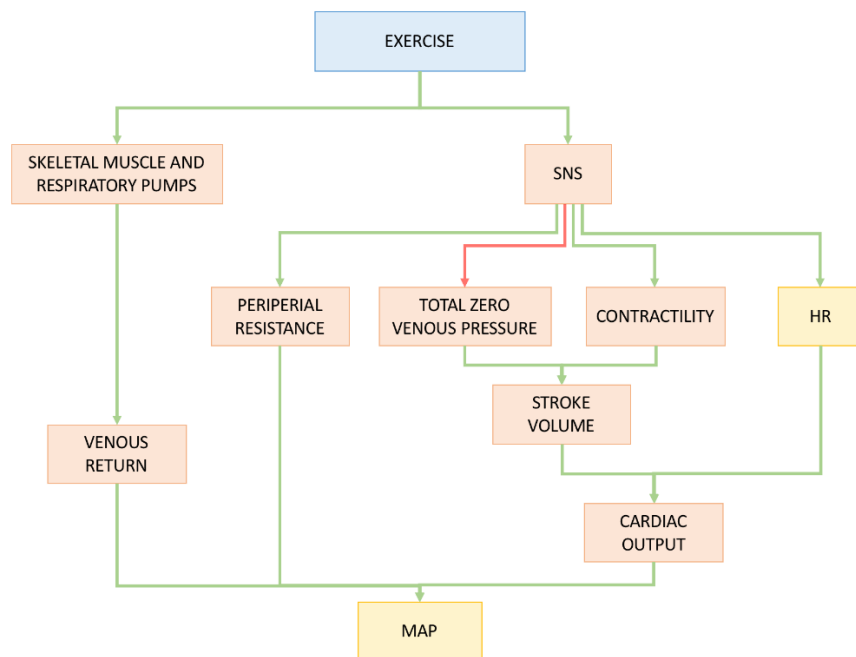


Figure 39. Block diagram showing the cardiovascular effects of exercise. Green lines represent a direct relationship while red ones represent an inverse relationship. Variables measured in our study are represented in yellow.

When studying the interaction of both factors together it is noteworthy the greater increase of heart rate during the exercise phase at lower artificial gravity levels. As we mentioned in the foot forces discussion, this response can be associated to the hypothetically greater VO_2 consumption at lower Ag. Higher VO_2 consumption necessarily implies higher cardiac output, which can be associated with higher HR. Interestingly, this interaction can also be seen at diastolic blood pressure, noticing a significantly increase when exercising in microgravity conditions. The causes of this sudden rise in DBP are still unknown and difficult to determine with the available data. We should also consider the blood pressure cuff measurements as a possible source of errors. Even if the LEM auscultatory blood pressure module was recommended for ergometry stress testing, when performing the exercise in microgravity configuration subjects had to maintain their position using the handlebars to not slide down the platform. The muscle contraction of the arms can potentially disturb the measurements and thus, should be considered as a possible reason of the DBP rise.

Bonjour proposed a quadratic function to fit the Heart Rate and Mean Arterial Pressure relationship with Ag using data available from other studies [53]. Using this model, he predicted HR and MAP responses at Mars and Moon gravity levels when cycling at 50W, 75W and 100W. The comparison between his predictions and the results obtained in this study are summarized in tables 13 and 14. When analyzing the responses at Earth's levels, we can observe our results are higher than the ones obtained by Bonjour comparing several centrifuge and space studies. The individual differences between the samples can likely explain this effect, suggesting that the subjects participating in our study were less physically prepared than the ones participating in the studies he used. It's also important to highlight that some of the studies he considered were performed on board the Russian Space Station and consequently, their subjects were part of the astronauts' population. However, qualitatively, Bonjour predictions fit pretty well our heart rate results with similar responses at Moon and Mars gravity levels as well as an increased heart rate under Earth conditions. In contrast, mean arterial pressure responses do not seem to follow any trend, oscillating between 103 and 112mmHg with no significant differences found between these three Ag conditions (i.e. Moon, Mars and Earth). Thus, we can say that we obtained a somewhat constant response similar to the predictions one (between 84 and 94 mmHg), but with higher values. Another important factor to consider is the difference in exposure time between the two studies; while our study only shows short term

adaptations, studies at 0g used by Bonjour to make the predictions were performed in space and therefore, involved long term adaptations of the body.

Table 13. Comparison between Bonjour's predictions of the HR responses to exercise on Moon and Mars with results obtained in this study.

HR (bpm)		50W	75W	100W
Moon	Prediction	87	96	105
	MAEPS study	113	124	137
Mars	Prediction	88	97	106
	MAEPS study	114	125	136
Earth	Prediction	93	102	112
	MAEPS study	118	131	141

Table 14. Comparison between Bonjour's predictions of the MAP responses to exercise on Moon and Mars with results obtained in this study.

MAP (mm Hg)		50W	75W	100W
Moon	Prediction	84	91	91
	MAEPS study	103	107	109
Mars	Prediction	85	92	92
	MAEPS study	109	111	112
Earth	Prediction	87	93	94
	MAEPS study	104	104	106

When interpreting blood pressure results another noteworthy the effect is the body weight in the anteroposterior direction induced by the simulation limitations. Buckey developed a model to determine the effects of the body weight on different pressures of the body, obtaining an increase of 7.6mmHg in central venous pressure in the supine position respect microgravity conditions [64]. If these simulation results are extrapolated to real 0g conditions, these effects need to be carefully quantified to not interfere with the predictions.

6.3. PULMONARY SYSTEM

The study showed a slightly increase of VO₂ with decreased gravity levels during the exercise phase, revealing a higher energy expenditure in microgravity conditions. This result may seem contradictory compared with previous studies which predicted a linear increase of VO₂ consumption with gravity [65]. Bonjour proposed a model where the metabolic consumption (\dot{E}) was positively linearly related with the Ag level (Equation 2).

$$\dot{E} = \dot{w} \cdot \Delta\eta^{-1} + \varepsilon \cdot M_L \cdot a_g \quad (2)$$

However, when looking at the foot forces data we observed an increased activation of the group of muscles involved in the pull-up at lower gravity conditions. As mentioned before, it is possible that this change in the pedaling technique could negatively affect the delta-efficiency of the exercise ($\Delta\eta$) [66] and consequently increase the energy expenditure at lower Ag level. According to that, the efficiency would be dependent on the artificial gravity level, $\Delta\eta(\text{Ag})$. It has to be noted that Stockholm's study mentioned before predicted VO₂ consumption at hyper-gravity levels as opposed to the hypo-gravity levels used in our study. Thus, differences between both can be explained by a more constant $\Delta\eta$ function at Ag levels greater than 1g. In fact, the efficiency changes here explained are due to the shift in the main muscle groups used, which occurs between 0g and 1g.

Bonjour also predicted VO₂ responses to exercise at hypo-gravity levels in an study comparing different experiments carried out at Buffalo, Stockholm, and inside the Mir Station [53]. A comparison between their predicted results for Mars and the Moon and the ones obtained at CAEPS are presented in table 15. The first difference is in regards to the magnitude of the results, with our results being much higher than the ones predicted by Bonjour. As we commented in the cardiovascular results' discussion, these results are probably due to individual differences. This makes sense given that some of the studies he considered included crew members which are more physically fit and thus, their VO₂ consumption levels are lower.

Another main difference is that they predicted a direct linear relationship between 0g and 1g while our data does not fit that assumption. We must highlight here that the model they obtained used spaceflights data for the hypo-gravity conditions and centrifuge data for the hyper-gravity ones, with no intermediate points. The main drawback of this procedure is that the body's adaptation is not the same with prolonged exposure to microgravity as it is with the acute responses of the centrifuge. In our study, microgravity data was obtained during an acute exposure and this difference could contribute to explain the different responses of VO₂ observed at hypo-gravity levels.

Table 15. Comparaison between Bonjour's predictions of the VO₂ responses to exercise on Moon and Mars with results obtained in this study.

VO ₂ (l/min)		50W	75W	100W
Moon	Prediction	0.74	0.99	1.29
	MAEPS study	1.44	1.68	1.98
Mars	Prediction	0.78	1.03	1.33
	MAEPS study	1.41	1.64	1.88
Earth	Prediction	0.87	1.12	1.42
	MAEPS study	1.39	1.60	1.80

As a response to the increased VO₂ demand, both VCO₂ and VE increased at lower gravity levels during the exercise phase. The analogy between VO₂ and VCO₂ responses highlights the reliability of our results. During aerobic respiration an increase in the quantity of oxygen inhaled immediately results in an increase in the carbon dioxide exhaled as well as an increase in total ventilation in order to meet the oxygen demand.

The way the pulmonary breathing mechanics adapted to achieve the new total ventilation included a slight increase in tidal volume and a decrease in the pulmonary rate with increased Ag levels. This selection of an increased tidal volume is presumably due to the increase in the thoracic pressure when being reclined [67]. The decrease in the tilt angle reduces the gravity force in the longitudinal axis of the subject but increases it in the direction perpendicular to the platform (i.e. the anteroposterior direction). As a consequence, there is a higher contribution of the weight forces of the thoracic cage and the lungs which is applied to the lungs themselves and thus makes their expansion more

difficult. It's important to note that this is considered a limitation of the simulation and we don't expect this to happen in real microgravity. These results match the ones obtained by Eliott during the Spacelab missions [27]. In concordance with our responses, a tidal volume reduction is observed when comparing supine posture with standing. This study also shows a decreased tidal volume when in space, however the mechanisms producing this response are unlikely to be the same that the ones acting during HDT simulations.

6.4. COMFORT AND STRENUOUSNESS DATA

This was the first experiment performed at the CAEPS platform. Microgravity conditions showed the lowest comfort punctuation with a 4.4 ± 2 over 10. The major complains reported were lower back pain and discomfort in the arms due to the handlebars. Future suggestions that could improve these issues include a better platform lining, intermediate cycle ergometer positions, and different handlebars configurations to enable a more ergonomic positioning of the subject on the platform. However, no cycling difficulties or major discomfort problems were reported, validating the correct functioning of the platform. Strenuousness results showed an increased perception of difficulty at lower artificial gravity levels. This result is probably related with the more unnatural positioning of the body but also seems to validate our previous hypothesis: 'Lower Ag levels induce a greater metabolic consumption'.

7. LIMITATIONS AND FUTURE WORK

In the process of designing an experiment, decision-making necessarily leads to some limitations of the study. In addition, some ideas that hadn't come up before appear during the experimental process and are postponed as a continuation of the study. Both limitations and future work of our study are listed below.

7.1. Limitations

Some of the main limitations of our study are:

- Few number of female subjects. Even if this lower female representation matches the astronaut population one, a higher number of female subjects would be desirable to allow the comparison of artificial gravity effects across gender.
- Body weight component in the anteroposterior direction of the body. When simulating partial-gravity using HDT and HUT this is the main drawback and its effects need to be quantified and removed from the results.
- Differences in body positions across different Ag conditions. The CAEPS design induced some differences between Earth and the other positions (Using a seat versus lying on a platform) and the effects have to be taken into account when interpreting the results.
- Use of handlebars., The CAEPS design required the use of handle bars to avoid sliding when performing exercise in one of the three reclined positions. However, its usage is not desirable since the activation of the arm muscles can interfere with the blood pressure measurements taken by the blood pressure cuff.
- Foot forces were only measured in the perpendicular direction. Due to the equipment available, tangential forces were not measured, which would have provided us with a more complete knowledge of the foot forces patterns.

- Cardiac output was not measured. Cardiac output, along with the cardiovascular variables measured (HR and MAP), would have enabled for a better overall picture of how the cardiovascular system works in different gravity conditions. However, due to the difficulties in measuring it using a non-invasive method ,its measurement was discarded.

7.2. Future work

Some future ideas to continue and expand the results of this study include:

- Generate dose-response curves of the studied cardiovascular and pulmonary variables using regression models to fit the obtained data.
- Deeper study of the acquired data including, for example, pulmonary kinetics or ventilatory thresholds.
- Adapt the cardiovascular lumped-parameter model developed at MIT [68] to simulate cardiovascular responses to tilt and use it to compare and validate the experimental results.
- Complete the actual results with future experiments measuring cardiac output.
- Use CAEPS platform in future studies to measure VO_{2max} in different altered-gravity environments.

8. CONCLUSIONS

The aim of this study was to shed some light on the big questions regarding artificial gravity. Some interesting results have been found including the increased metabolic consumption when cycling at lower gravity levels. This result suggests a higher activation of the cardiovascular and pulmonary systems when exercising in microgravity. Combining artificial gravity with exercise seems to play here an excellent role in mitigating the detrimental effects of weightless.

Another particularly interesting finding was the similarity in the multi-system physiological response under Martian and terrestrial gravitational environments. This discovery implies similar cardiopulmonary and musculoskeletal stimuli on both planetary surfaces, suggesting the potential for lower physiological deconditioning on Mars.

Finally, the experiment was successfully completed at CAEPS, validating its potential in simulating different altered-gravity environments using tilt angles while performing cycling ergometer exercise. Feedback of the subjects also helped determine the main drawbacks of its design and will enable future improvements.

Much more knowledge is still needed in this area to fully understand how a system as complex as our body adapts to changes in gravity. However, the results obtained from this study will contribute a small piece to the vast and intricate puzzle of artificial gravity.

APPENDIX: FORMS OF THE EXPERIMENT

IRB APPROVAL



Cornell University
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Institutional Review Board for Human Participants

NOTICE OF EXPEDITED APPROVAL

To: Ana Diaz Artilles
From: Carol Devine, IRB Chairperson
Protocol ID#: 1706007254
Protocol Title: Exercise in Altered-Gravity Environments
Approval Date: September 29, 2017
Expiration Date: September 28, 2018

A handwritten signature in black ink, reading 'Carol M. Devine'.

Cornell University's Institutional Review Board for Human Participants (IRB) has reviewed and approved the inclusion of human participants in the research activities described in the protocol referenced above. This approval shall remain in effect until **September 28, 2018**.

The following personnel are approved to perform research activities on this protocol:

- Ana Diaz Artilles
- Evan Halloran
- Francisca Perez
- Patricia Navarro

This approval by the IRB means that human participants can be included in this research. However, there may be additional university and local policies that apply before research activities can begin under this protocol. It is the investigator's responsibility to ensure these requirements are also met.

Please note the following important conditions of approval for this study:

1. All consent forms, records of study participation, and other consent materials **must** be held by the investigator for **five years** after the close of the study.
2. Investigators must submit to the IRB any **proposed amendment** to the study protocol, consent forms, interviews, recruiting strategies, and other materials. Investigators may not use these materials with human participants until receipt of written IRB approval for the amendment. For information about study amendment procedures and access to the Amendments application form, please refer to the IRB website: <http://www.irb.cornell.edu/forms>.

CONSENT FORM

Exercise in Altered-Gravity

Consent to Participate in Research

Exercise in Altered-Gravity

You are being asked to participate in a research study conducted by Dr. Ana Diaz Artilles from the Sibley School of Mechanical and Aerospace Engineering at Cornell University. We are asking you to take part in this study because you volunteered and meet the health and physical requirements to participate. Please read the form carefully and ask any questions you may have before agreeing to take part in the study.

Purpose of the Study

Astronauts on space missions experience various detrimental physiological effects, mostly due to the weightless environment. Exercise provides a potential countermeasure to several of these negative health effects, particularly when combined with exposure artificial gravity. The purpose of this study is to determine the physiological effects that exercise in altered gravity environments has on the human body in order to gain a better understanding of how to combat deconditioning in space.

Procedures

If you agree to participate in the study, you will take part in four different exercise sessions on the Microgravity Exercise Performance Simulator (MEPS). Each study session will be no longer than 1 hour within which you will complete an exercise protocol corresponding to a specific simulated gravity environment. Before the exercise protocol you will be briefed on disqualifying medical conditions and given a pre-participation questionnaire to determine eligibility for the study. You will then be briefed on the function of the MEPS, the various data collection devices, and the experimental protocol. Data collection devices include a tape measure, scale, thermometer, heart rate monitor, cardiovascular monitor, electromyography sensor, foot sensors, and cardiopulmonary exercise testing equipment. The MEPS will also collect data such as revolutions per minute (RPM) and distance traveled. You will also be asked questions about your comfort level periodically throughout the exercise protocol. After the briefing, we will take your height and weight and ask for your age and gender.

For the testing, you will lay supine on the angled table for three of the exercise protocols and sit upright on the bike for one protocol. Your feet will be strapped to the pedals and the various sensors will be attached. Throughout the protocol you will be subject to various levels of resistance from the bicycle which will require various levels of physical exertion, never exceeding 100 RPM. You will be asked questions about your comfort and fatigue levels throughout the protocol and will be given directives by the experimenter if your pedaling speed should be adjusted.

After the protocol the various sensors and foot straps will be removed and you will be removed from the MEPS. You will be given a short exit survey to fill out regarding your comfort levels and experience throughout the study.

Risks and Benefits

There is risk that you may get injured throughout the exercise protocol while on the exercise bike, but this is very rare. You may feel strain and fatigue during and after the exercise protocol.

In order to mitigate risk of injury, subject comfort has been a primary driver in the design of the platform and cycle hardware. The researcher will work with you prior to all trials to fine tune the testing configuration to minimize discomfort. The experimenter will frequently ask you questions to ensure comfort and check fatigue levels, and your alertness and wellbeing will be monitored continuously during trials. Testing will end immediately if you feel the need to stop the exercise at any time for any reason.

There are no direct benefits to you for participating in this study.

In Case of Injury

It is highly unlikely that injury will result from participation in this research. However, in the event that any study-related activities result in an injury, treatment will be made available including first aid and referral for emergency care as needed. Cost for such care will be billed in the ordinary manner to you or your insurance company. Emergency medical care is not available on-site. No reimbursement, compensation, or free medical care is offered by Cornell University. If you think that you have suffered an injury related to your participation in this study, contact Dr. Ana Diaz Artiles.

If there is a need for immediate medical attention, seek evaluation by your primary care provider or the emergency room.

Compensation

You will not be given compensation for participation in this study.

Confidentiality

All of your information from the study will remain confidential and all data taken from the experiment will remain on secure computers in a locked lab. Only researchers will have access to the data from the study. If any reports or publications from the experiment are produced, no information that could be used to identify you will be released.

Data Sharing

De-identified data from this study may be shared with the research community at large to advance science and health. We will remove or code any personal information that could identify you before files are shared with other researchers to ensure that, by current scientific standards and known methods, no one will be able to identify you from the information we share. Despite these measures, we cannot guarantee anonymity of your personal data.

Taking Part is Voluntary

Taking part in this study is completely voluntary. If at any time you wish to skip or stop a portion of the experiment you may do so with no penalty and it will not affect your

Exercise in Altered-Gravity

current or future relationship with Cornell University. If you choose to take part in the study, you may withdraw at any time.

Questions

The researchers conducting this study are Dr. Ana Diaz Artiles and the qualified students working on this research project. Please ask any questions you have now. If you have questions later, the contact information for the researchers is listed below. If you have questions or concerns regarding your rights as a subject in this study, you may contact the Institutional Review Board (IRB) at 607-255-5138 or access their website at <http://irb.cornell.edu>. You may also report your concerns or complaints anonymously through Ethicspoint (www.hotline.cornell.edu) or by calling their toll free number at 1-866-293-3077. Ethicspoint is an independent organization that serves as a liaison between the University and the person bringing the complaint so that anonymity can be ensured.

Principal Investigator Dr. Ana Diaz Artiles 463 Upson Hall Ithaca, NY 14853 (607) 255-3249 ad877@cornell.edu
--

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information, and have received answers to any questions I asked. I consent to take part in the study.

Your Signature _____

Date _____

Your Name (printed) _____

Signature of person obtaining consent _____

Date _____

Printed Name of person obtaining consent _____

Date _____

This consent form will be kept by the researcher for at least three years beyond the end of the study.

QUESTIONNAIRES

Exercise in Altered-Gravity

Eligibility and Pre-Participation Questionnaire

MEPS Orientation: _____

Date: _____

Please circle "Yes" or "No" for each of the following questions. For any questions to which you answered, "Yes", please briefly explain. Answering "Yes" does not automatically disqualify you from the study.

Yes No 1. Do you currently have any neck, back, chest, hip, knee, ankle, or foot pain/discomfort?

Yes No 2. Are you currently using any cardiac, blood pressure, or muscle relaxation/stimulant medications?

Yes No 3. Do you have any issue doing 1 hour of cardiovascular/aerobic exercise?

Yes No 4. Do you exercise regularly?
If so, what form (circle)? Cardiovascular Strength training
How many days per week? _____
How many hours per session? _____
Describe your typical routine (which exercises, weights/reps, etc.)

Yes No 5. Are you, or could you, be pregnant?

Exercise in Altered-Gravity

Yes No 6. Do you have any known thyroid issues?

Yes No 7. Do you have any history of any condition of the heart, lungs, central nervous system, metabolic or endocrine system, bones or orthopedic system, or reproductive system, or other health issue, that might be adversely affected by performing 40 minutes of vigorous exercise?

TO BE FILLED OUT BY INVESTIGATOR

Subject Number: _____

Weight: _____

Height: _____

Age: _____

Gender: _____

Target HR: _____

Exercise in Altered-Gravity

Exit Survey

1. On a scale of 1-10 how comfortable was cycling on the MEPS and how natural did it feel (1=very uncomfortable/unnatural, 10=very comfortable/natural)? What, if any, components contributed to discomfort?

2. On a scale of 1-10 how strenuous do you feel the exercise regimen was (1=easy, 10=very strenuous)? If strenuous, was this because of the resistance on the exercise device, the duration of the exercise, or both?

3. Do any muscles or areas of your body feel sore?

4. Did you notice any discomfort due to your orientation on the platform?

5. Any other comments you'd like to share?

FOR CLARIFICATION ONLY, THIS PAGE NOT GIVEN TO SUBJECTS

Medical Screening

Subjects will be screened for medical conditions by the Eligibility/Pre-Participation Questionnaire. Of the 7 questions, there are five for which a “yes” answer would result in an automatic disqualification (#1, 2, 3, 5, 6) as these indicate the subject has a current medical condition or is on a medication that puts them at risk, or may not be capable of completing the exercise protocol.

In question 7 a “yes” answer would result in disqualification depending on the time period since the indicated injury. If subjects indicate the injury has occurred within the last 6 months they will be disqualified, otherwise they will be allowed to participate.

Question 4 pertains to the subject’s exercise routine. This question is for data collection purposes and does not affect the subject’s eligibility.

PROTOCOL CHECKLIST

Subject:

MEPS Orientation:

Date:

Time:

Protocol Checklist

A. Setup

- Power on test computer (TC)
 - Username: DiazLab
 - Password: microgravity
- Connect the K4b² Unit to power and allow system to warm up for 45 min
- Connect blood pressure apparatus
- Connect and power on ergometer (*Use RS232in port on Angio*)
- TC: Open LEM
 - a. Login
 - Username: lode
 - Password: service
 - b. Run connection test on ergometer (prints "Communication Successful")
 - Device name: Angio 2003 (B)
 - Communication port: COM1
 - Serial: 20120505
 - Serial Protocol: Lode 38k4
- Connect LabQuest Mini to TC and devices
 - Ch1 = Foot Force 1 (left foot)
 - Ch2 = Foot Force 2 (right foot)
- TC: Open Logger Lite
 - a. Zero foot sensors
 - i. Hold pedals in the highest position (90°) with the base of the plate perpendicular to ground
 - ii. In Logger: Experiment -> Zero

- b. Ensure that sensors produce accurate readings after zeroing
- c. Check and write sensor inputs:
 - i. Force 1 (left foot): _____
 - ii. Force 2 (right foot): _____
- d. Check collection frequency and recording duration (In Logger: Experiment -> Data Collection)
 - Duration: 45 min
 - Sampling Rate: 750 samples/min
- e. File/Save As: logger_subject_position

- TC: Open K4b2 software
- Ensure platform is in desired position and anchored
- Ergometer baseplate.....LOCKED
- Handles (if applicable).....LOCKED
- Seat (if applicable).....LOCKED
- Record room temperature and humidity
 - Temperature: _____
 - Humidity: _____
- Once K4b² has warmed up, perform the Turbine Calibration, Room Air Calibration, and Gas Calibration per attached instructions
- Prepare consent form, eligibility questionnaire, and post-protocol survey

B. Subject Orientation

- Review consent form with subject
- Sign consent form
- Administer eligibility questionnaire

- Check results of questionnaire with medical screening requirements to determine eligibility of subject
- Check subject's clothing (nothing loose, close-toed shoes, NO GARMIN, etc.)
- Get subject's information:

Name: _____

Weight: _____

Height: _____

Age: _____

Gender: _____

DOB: _____
- Get a subject ID: _____
- Enter data in LEM profile
- Enter data in K4b2 software
- Perform Delay Calibration with subject for K4b² unit

C. Boarding

- Have subject wash hands with soap and water for minimum of 20 seconds
- Offer the subject a glass of water.
- Assist subject in laying on platform with pillow under head
- Adjust bike to fit subject

	Lower					Higher				
Seat	1	2	3	4	5	6	7	8	9	10
Handlebars	1		2		3		4		5	
Ergometer	1		2		3		4		5	

- Allow subject to don HR chest belt
- Attach the chest harness to the subject, inserting the K4b² Portable Unit in the holder with the flowmeter and power cord attached
- Don the facemask and head strap onto the subject, attaching the flowmeter to the facemask once secured
- Connect the K4b² Unit to TC
- Attach blood pressure cuff to right arm of subject
- Assist subject in sitting with hands on knees

D. Preparation for Data Collection

- Configure LEM for data collection
 Test Subject: _____
 Test Protocol: Testing Protocol
 Test Ergometer: Angio 2003 (B)
 Visualization: workload, rpm, protocol, blood pressure
- LEM: Click start and wait for “Initialization Successful” message
 - a. If problem, check that bike is on and powered
- Configure K4b2 for data collection
 - a. Select subject (File -> Patients -> List)
 - b. Click Test and ensure that HR produce accurate readings

E. Trial

- Start data collection simultaneously:
 Time: _____
 a. LEM: “Testing Protocol” Protocol

- b. Logger Lite
 - c. K4b² Software
- Allow subject to stay in sitting position for: 5 min
- Assist subject in laying on platform with pillow under head
- Strap subject's feet onto pedals
- Allow subject to lay in this position for: 7 min
- Start the metronome at 180 bpm
- Alert subject to adjust position and rpm as needed. At 5 min intervals ask about comfort and positioning of:
 - a. Ergometer
 - b. Facemask and head strap
 - c. Arm cuff
 - d. Chest strap
 - e. Foot straps
- Check sensors for accurate readings throughout protocol
 - a. LEM
 - b. Logger Lite
 - c. K4b²
- Throughout trial alert subject to upcoming changes in workload
- At the end of the exercise protocol, tell subject to stop cycling
- Let the subject rest on the platform for 7 min
- Time at end: _____
- Stop/Save LEM protocol
- Stop/Save Logger Lite data
- Stop/Save K4b² data

F. Post-Trial

- Unstrap subject:
 - a. Foot straps
 - b. Blood pressure cuff
 - c. Facemask and head strap
 - d. Chest harness
- Allow subject to remove chest strap
- Administer exit survey
- Export LEM data and exit the software
- Export Logger Lite data and exit the software
- Export K4b² Unit data and exit the software
- Power off ergometer and disconnect
- Power off and disconnect metabolic equipment
- Disconnect LabQuest and accompanying sensors
- Power Off TC

G. Cleaning

- Clean mat, handlebars, and seat (if applicable) with disinfecting wipe
- Cover pillow with plastic sheath
- Disinfect HR chest belt and wristband with disinfecting wipe
- Disinfect blood pressure apparatus with wipe
- Disinfect K4b² facemask and flowmeter per attached instructions

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